LIVER FIBROSIS:
THE NEXT GOAL OF
TARGETED THERAPY?

17-18 JUNE 2016
PORTO, PORTUGAL

Scientific Committee
Sophie Lotersztajn, France
Massimo Pinzani, United Kingdom
Christian Trautwein, Germany

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Dear Colleagues,

On behalf of the EASL, The Home of Hepatology, we are delighted to welcome you to Porto for the EASL Monothematic Conference ‘Liver Fibrosis: the next goal of targeted therapy?’.

More than 30 years of intense research activity on this topic has brought major advances in the understanding and clinical management of the fibrogenic process common to most chronic liver diseases. There are however many aspects that certainly deserve further attention. Our conference is dedicated to all the current key areas of research in liver fibrogenesis and will focus on a number of open issues requiring further scientific efforts. The role of genetic/epigenetic factors and the immune system, and the reversibility of fibrosis and methodologies for the identification of more reliable targets for drug development will represent some of the hot topics to be covered.

Young scientists are particularly encouraged to participate and will have the opportunity to present their original results during a dedicated session. The format is intended to generate active interactions and discussion between basic scientists and clinicians, and to foster future collaborative efforts to better understand the pathogenesis of tissue fibrosis in chronic liver disease. Ultimately, to improve patient management.

We sincerely hope that you will enjoy our conference and have a great time in Porto.

With warm regards,

Dr Sophie Lotersztajn
Paris, France

Prof. Massimo Pinzani
London, United Kingdom

Prof. Christian Trautwein
Aachen, Germany
TOPICS TO BE COVERED
Fibrosis
Cirrhosis
Etiology
Genetics and Epigenetics
Immunity
Inflammation
Disease modelling
Therapeutics approaches

TARGET AUDIENCE
Hepatologists
Physicians with an interest in Hepatology
Basic scientists
Health professionals
Young investigators and trainees

WHY ATTEND?
2 days of cutting edge clinical and basic research on Fibrogenesis
The latest on NASH diagnosis and management
8 sessions covering the latest on Fibrosis and Cirrhosis diagnosis and management
Gather and network with renowned top scientists and specialists
Share and discuss your latest results
SCIENTIFIC COMMITTEE

Dr Sophie Lotersztajn, Paris, France
Prof. Massimo Pinzani, London, United Kingdom
Prof. Christian Trautwein, Aachen, Germany

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ACKNOWLEDGEMENTS

PREMIUM SPONSORS

EASL thanks its Premium Sponsors for their generous contributions and support of the EASL Monothematic Conference ‘Liver fibrosis: The next goal of targeted therapy?’ with an unrestricted educational grant.

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GENERAL INFORMATION
GENERAL INFORMATION

CONFERENCE VENUE
Porto Palacio Congress Hotel & Spa
Av. da Boavista 1269 4100-130,
Porto, Portugal

DISCOVER PORTO
City website: http://www.visitporto.travel

Porto is one of Europe’s oldest touristic destinations. Its wealth of artistic heritage, Port Wine, open-air leisure spaces and cultural life are just some of the reasons to visit.

The city unfolds along the river bank and the sea shore to reveal charming vistas, inviting esplanades and all the pleasures of the outdoors, framed by green spaces. Discovering Porto means encountering surprise after surprise. Whilst maintaining its welcoming and conservative nature, the city is also contemporary and creative. This can be seen in its streets, architecture, museums, leisure spaces, esplanades, and shopping areas which run from the traditional to the modern and exclusive.

LANGUAGE
The official language of the conference is English.

CLIMATE
The average temperature in June is 18°C (64°F), so we expect to have plenty of sunshine and only an occasional shower to keep the atmosphere fresh and humidified. It is however known for temperatures to rise to around 22°C particularly in the third and fourth week towards July.

NAME BADGES
All participants are kindly requested to wear their name badges throughout the EASL Monothematic Conference in order to be admitted to the lecture halls and other scheduled activities.

REGISTRATION AND ACCOMMODATION
All participants are invited to register online in order to save time upon their arrival at the conference.

Hotel accommodation for the EASL Monothematic Conference will be offered to participants during the online registration process. Detailed information, as well as access to the online registration is available on www.easl.eu. Registered participants are entitled to reduced conference hotel rates.

REGISTRATION DESK
The onsite registration desk at the conference venue will be open at the following times:

Thursday
16 June 2016 from 16:00 to 20:00

Friday
17 June 2016 from 08:00 to 18:30

Saturday
18 June 2016 from 08:30 to 13:30
CME ACCREDITATION
The ‘EASL Monothematic Conference – Porto, Portugal, 17-18 June 2016’ is accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide the following CME activity for medical specialists. The EACCME is an institution of the European Union of Medical Specialists (UEMS), www.uems.net.

The ‘EASL Monothematic Conference – Porto, Portugal, 17-18 June 2016’ is designated for a maximum of (or ‘for up to’) 10 hours of European external CME credits. Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity.

Through an agreement between the European Union of Medical Specialists and the American Medical Association, physicians may convert EACCME credits to an equivalent number of AMA PRA Category 1 Credits™. Information on the process to convert EACCME credit to AMA credit can be found at www.ama-assn.org/go/internationalcme.

Live educational activities, occurring outside of Canada, recognized by the UEMS-EACCME for ECMEC credits are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of The Royal College of Physicians and Surgeons of Canada.

EACCME CREDITS
Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity. The EACCME credit system is based on 1 ECMEC per hour with a maximum of 3 ECMECs for half a day and 6 ECMECs for a full-day event.

FLIGHTS TO PORTO
Flights are available from the following airports:

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<tr>
<th>BELGIUM</th>
<th>FRANCE</th>
<th>GERMANY</th>
<th>IRELAND</th>
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TRANSPORT TO THE VENUE
The conference venue, Porto Palacio Congress Hotel & Spa, is 17 Km or 20 minutes from Francisco Sá Carneiro Airport (OPO).

By train
7 Km from Campanha station.

By car
Take the A1 motorway and leave at the first exit after Arrábida Bridge (Bessa Leite). The Porto Palacio Congress Hotel is situated on the right-hand side of Avenida da Boavista.

By underground
The Porto Palacio Congress hotel is a 10-minute walk from the Casa da Música underground station.

PARTICIPANTS’ LIST
The participants’ list will be displayed and located onsite at the EASL booth.

DRESS CODE AND SMOKING POLICY
Dress code is informal for all occasions. This will be a non-smoking event.

BANKING, SAFETY AND SECURITY
The currency used in Portugal is the EURO. Foreign currency can be exchanged at banks, bureau de change, and automatic currency exchange machines.

Please do not leave bags or suitcases unattended at any time, whether inside or outside the session halls. Hotels strongly recommend that you use their safety deposit boxes for your valuables.

LIABILITY AND INSURANCE
The EASL Office cannot accept liability for personal accidents or loss of, or damage to, private property of participants. Participants are advised to take out their own personal travel and health insurance for their trip.
SCIENTIFIC PROGRAMME
DAY 1 – FRIDAY 17 JUNE 2016

08:30 – 09:00  Registration and ePoster presentations 1

1. SHAPING LIVER FIBROSIS: THE IMPACT OF ETIOLOGY, GENETIC AND EPIGENETIC FACTORS

Chairs: Christian Trautwein, Germany  
       Jelena Mann, United Kingdom

09:00 – 09:20  FROM WOUND-HEALING TO FIBROSIS  
               Thomas Wynn, United States

09:20 – 09:40  IMPACT OF ETIOLOGY ON LIVER FIBROSIS  
               Pierre Bedossa, France

09:40 – 10:00  IMPACT OF GENETIC ON LIVER FIBROSIS  
               Frank Lammert, Germany

10:00 – 10:20  IMPACT OF EPIGENETICS ON LIVER FIBROSIS  
               Jelena Mann, United Kingdom

10:20 – 10:50  ePoster presentations 2 and coffee break

2. MODULATING LIVER FIBROSIS: THE ROLE OF INNATE AND ACQUIRED IMMUNITY

Chairs: Sophie Lotersztajn, France  
       Jonathan Fallowfield, United Kingdom

10:50 – 11:10  MICROBIOME AND FIBROSIS PROGRESSION  
               Christian Trautwein, Germany

11:10 – 11:30  THE ROLE OF MACROPHAGES IN LIVER FIBROSIS  
               Frank Tacke, Germany

11:30 – 11:50  INNATE LYMPHOID CELLS IN LIVER INFLAMMATION, FIBROSIS AND REPAIR  
               Veronika Lukacs-Kornek, Germany
11:50 – 12:10  **LYMPHOCYTE-ENDOTHELIAL INTERACTIONS: WHY ARE THEY IMPORTANT?**  
Patricia Lalor, *United Kingdom*

**12:10 – 13:00 Lunch break**

**12:30 – 13:00**  
*ePoster presentations 3*

**3. INFLAMMATION AND FIBROSIS: ALWAYS A LINEAR RELATIONSHIP?**

*Chairs: Thomas Wynn, United States*  
*Frank Lammert, Germany*

**13:00 – 13:20**  
**FRAMING THE INFLAMMASOME**  
Alexander Wree, *Germany*

**13:20 – 13:40**  
**ADIPOKINES AND FIBROSIS**  
Fabio Marra, *Italy*

**13:40 – 14:00**  
**ANGIOGENESIS AND LIVER FIBROSIS**  
Vijaj Shah, *United States*

**14:00 – 14:20**  
**IMMUNOREGULATION OF LIVER FIBROSIS: NOVEL PLAYERS**  
Sophie Lotersztajn, *France*

**14:20 – 14:40**  
**ACTIVATION OF PROGENITOR CELLS IN LIVER FIBROSIS**  
Pau Sancho-Bru, *Spain*

**14:40 – 15:10**  
*ePoster presentations 4 and coffee break*

**4. CIRRHOSIS: IN SEARCH OF A BETTER DEFINITION**

*Chairs: Tilman Sauerbruch, Germany*  
*Jessica Zucman-Rossi, France*

**15:10 – 15:30**  
**CIRRHOSIS OR CIRRHOSES?**  
Massimo Pinzani, *United Kingdom*

**15:30 – 15:50**  
**LIVER FIBROSIS AND PORTAL HYPERTENSION**  
Jonel Trebicka, *Germany*
### 15:50 – 16:10  
**CIRRHOSIS AS A SYSTEMIC DISEASE**  
Rajiv Jalan, United Kingdom

### 16:10 – 16:30  
**CIRRHOSIS AS A PRE-NEOPLASTIC CONDITION**  
Jessica Zucman-Rossi, France

### 5. REGRESSION OF FIBROSIS AND CIRRHOSIS

**Chairs:** Jonel Trebicka, Germany  
Rajiv Jalan, United Kingdom

<table>
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<tr>
<th>Time</th>
<th>Session</th>
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| 16:30 – 16:50 | **FIBROSIS AND CIRRHOSIS REGRESSION: CLINICAL IMPLICATIONS (THE POST HCV SCENARIO)**  
Antonio Craxi, Italy |
| 16:50 – 17:10 | **THE CELLULAR AND MOLECULAR COMPONENTS OF FIBROSIS REGRESSION**  
Jonathan Fallowfield, United Kingdom |
| 17:10 – 17:30 | **HOW TO ASSESS REGRESSION: PATHOLOGY?**  
Amar Paul Dhillon, United Kingdom |
| 17:30 – 17:50 | **HOW TO MONITOR REGRESSION: NON INVASIVE METHODS?**  
Laurent Castera, France |

### 17:50 – 18:20  
ePoster presentations 5

### 17:50  
Cocktail reception
### DAY 2 – SATURDAY 18 JUNE 2016

#### 08:30 – 09:00  
*EPoster presentations 6 and registration*

#### 6. ADVANCES IN IMAGING AND DISEASE MODELLING

**Chairs:** Detlef Schuppan, *Germany*  
Massimo Pinzani, *United Kingdom*

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<th>Time</th>
<th>Session</th>
<th>Speaker</th>
<th>Country</th>
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| 09:00 – 09:20 | **Molecular Magnetic Resonance Imaging of Liver Fibrosis in Animal Models**  
**NEW INVITRO MODELS TO INVESTIGATE FIBROSIS – 3D VS 2D**  
**Critical Discussion of Mouse Models for Liver Fibrosis** | Peter Caravan       | United States |
|               |                                                                         | Krista Rombouts    | United Kingdom|
|               |                                                                         | Robert Schwabe     | United States |
| 10:00 – 10:30 | *EPoster presentations 7 and coffee break*                             |                    |               |

#### 7. SELECTED ABSTRACTS PRESENTATION

**Chairs:** Fabio Marra, *Italy*  
Veronika Lukacs-Kornek, *Germany*

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<th>Time</th>
<th>Session</th>
<th>Speaker</th>
<th>Country</th>
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<tbody>
<tr>
<td>10:30 – 10:45</td>
<td><strong>Progressive Fibrosis Is Driven by Genetic Predisposition, Allo-Immunology, and Inflammation in Pediatric LT Recipients</strong></td>
<td>Sharat Varma</td>
<td>Belgium</td>
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<tr>
<td>10:45 – 11:00</td>
<td><strong>Relaxin-Coated Iron-Oxide Magnetic Nanoparticles as a Novel Theranostic Approach for Diagnosis and Treatment of Liver Fibrosis</strong></td>
<td>Ruchi Bansal</td>
<td>Netherlands</td>
</tr>
<tr>
<td>11:00 – 11:15</td>
<td><strong>IL-13 Drives Distinct Cellular Pathways Directing Ductular Reaction, Steatosis, and Fibrosis</strong></td>
<td>Richard Gieseck III</td>
<td>United States</td>
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</tbody>
</table>
11:15 – 11:30  THE I148M PNPLA3 VARIANT AS NOVEL KEY PLAYER MODULATING THE PRO-FIBROGENIC PHENOTYPE OF HUMAN HEPATIC STELLATE CELLS
Francesca Bruschi, Austria

11:30 – 11:45  GAS6/MERTK PATHWAY AS NEW PLAYER IN LIVER FIBROSIS PROCESS
Giovanni Di Maira, Italy

11:45 – 12:00  CIRCULATING ENDOTHELIAL PROGENITOR CELLS OF CIRRHOTICS ENHANCE ANGIOGENESIS AND LIVER FIBROSIS IN BILE DUCT-LIGATED RATS
Mohsin Hassan Bhat, India

12:00 – 12:15  THERAPEUTIC INTERVENTION WITH EVASIN-4 ATTENUATES LIVER FIBROGENESIS AND THE PROGRESSION OF HEPATOCELLULAR CARCINOMA
Antje Mohs, Germany

12:15 – 13:30  Lunch break

8. THERAPEUTIC APPROACHES AND TRIAL DESIGNS

Chairs: Massimo Pinzani, Italy
Christian Trautwein, Germany

13:30 – 13:50  TARGETING FIBROSIS INVIVO
Derek Mann, United Kingdom

13:50 – 14:10  CLINICAL GRADE EXPANDED LIVER DERIVED STEM CELLS FOR FIBROSIS & INFLAMMATION
Etienne Sokal, Belgium

14:10 – 14:30  HOW TO TRANSLATE BASIC CONCEPTS TO TRIAL END-POINTS?
Detlef Schuppan, Germany

14:30 – 14:50  IDEAL STUDY DESIGNS FOR ANTIFIBROTIC THERAPIES AND CURRENT INDUSTRY ACTIVITIES
Scott Friedman, United States
ePOSTER PRESENTATIONS
# DAY I – FRIDAY 17 JUNE 2016

**Session I**  
ePoster presentations 08:30 – 09:00

<table>
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<tr>
<th>Screen</th>
<th>Title</th>
<th>Abstract</th>
<th>Presenter</th>
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<tbody>
<tr>
<td>1</td>
<td>Interleukin-33/interleukin-33r signaling promotes liver inflammation and fibrogenesis in obesogenic model of nonalcoholic steatohepatitis in mice</td>
<td>MP-186</td>
<td>Nemanja Jovicic</td>
</tr>
<tr>
<td>2</td>
<td>Relationship between ca19.9 and fibrosis in a cohort of patients with viral hepatitis and without malignancies. Ca19.9 levels reflect the gravity of fibrosis</td>
<td>MP-198</td>
<td>Emanuele Crisafulli</td>
</tr>
<tr>
<td>3</td>
<td>Galectin-3 plays an important role in early stages of non-alcoholic steatohepatitis by promoting activation of natural killer cells</td>
<td>MP-205</td>
<td>Ilija Jeftic</td>
</tr>
<tr>
<td>4</td>
<td>Albi score as non-invasive biomarker of cirrhosis in chronic hepatitis c</td>
<td>MP-171</td>
<td>Ángel Hernández-Bartolomé</td>
</tr>
<tr>
<td>5</td>
<td>Efficacy of t1 mapping on gd-eob-dtpa-enhanced mri for staging liver fibrosis in chronic hepatitis b patients with normal alanine transaminase ≤ 40 iu/l</td>
<td>MP-181</td>
<td>LiYang</td>
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<tr>
<td>6</td>
<td>Rnai-mediated inhibition of cyclin e1 protects from liver fibrosis in mice</td>
<td>MP-183</td>
<td>Roland Sonntag</td>
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### Session 2
**ePoster presentations 10:20 – 10:50**

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<tr>
<th>Screen</th>
<th>Title</th>
<th>Abstract</th>
<th>Presenter</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Surprising pro-fibrotic effect of macrophage migration inhibitory factor in a methionine-choline deficient diet model is associated with a shift in natural killer t cell populations</td>
<td>MP-123 [Daniel Heinrichs]</td>
<td></td>
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<tr>
<td>2</td>
<td>The glp-1 receptor agonist liraglutide promotes deactivation of hepatic stellate cells leading to a marked improvement in the liver microvascular dysfunction of rats with chronic liver disease</td>
<td>MP-138 [Jordi Gracia-Sancho]</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>The platelet-derived chemokine cxcl4 exerts protective role in non-alcoholic steatohepatitis (nash) in vivo</td>
<td>MP-145 [Hannah Drescher]</td>
<td></td>
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<tr>
<td>4</td>
<td>The unfolded protein response is a very early event during hepatic stellate cell activation</td>
<td>MP-160 [Inge Mannaerts]</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>The role of necroptosis in chronic cholestasis-induced fibrosis</td>
<td>MP-178 [Marta Afonso]</td>
<td></td>
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<tr>
<td>6</td>
<td>Arterial pressure suffices to increase liver stiffness</td>
<td>MP-141 [Sebastian Mueller]</td>
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## Session 3
### ePoster presentations 12:30 – 13:00

<table>
<thead>
<tr>
<th>Screen</th>
<th>Title</th>
<th>Abstract</th>
<th>Presenter</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Prediction of post-operative liver failure in cirrhotics undergoing surgical resection for hepatocellular carcinoma: the role of liver stiffness measured by acoustic radiation force impulse and the proposal for a new mathematical model score</td>
<td>MP-208</td>
<td>Marinella Lupo</td>
</tr>
<tr>
<td>2</td>
<td>Platelet specific junctional adhesion molecule-a deficiency attenuates experimental liver fibrosis in mice</td>
<td>MP-185</td>
<td>Theresa Wirtz</td>
</tr>
<tr>
<td>3</td>
<td>Diverse roles of amine oxidases in liver fibrosis</td>
<td>MP-187</td>
<td>Emma Shepherd</td>
</tr>
<tr>
<td>4</td>
<td>Activation of cebp by oligonucleotide sarna therapy in progressive liver failure reverses liver fibrosis and promotes liver regeneration</td>
<td>MP-193</td>
<td>Vikash Reebye</td>
</tr>
<tr>
<td>5</td>
<td>Liver fibrosis in correlations with cd4/cd8 and platelet count in patients with chronic hepatitis delta</td>
<td>MP-102</td>
<td>Adela Turcanu</td>
</tr>
<tr>
<td>6</td>
<td>Fibroscan versus liver biopsy in the evaluation of response among the Egyptian hcv infected patients to treatment</td>
<td>MP-104</td>
<td>Dina Ziada</td>
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### Session 4
**ePoster presentations 14:40 – 15:10**

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<th>Screen</th>
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<th>Abstract</th>
<th>Presenter</th>
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<tbody>
<tr>
<td>1</td>
<td>Fishing for novel targets of liver fibrosis by using danio rerio model</td>
<td>MP-132</td>
<td>Michal Pawlak</td>
</tr>
<tr>
<td>3</td>
<td>Validation of elf test score for hepatic fibrosis evaluation in turkish chronic liver disease patients</td>
<td>MP-111</td>
<td>Osman Ozdogan</td>
</tr>
<tr>
<td>4</td>
<td>Antifibrogenic effect of statins used for treatment of non-alcoholic steatohepatitis (nash)</td>
<td>MP-117</td>
<td>Andra Suceveanu</td>
</tr>
<tr>
<td>5</td>
<td>Does elevated sinusoidal pressure cause liver cirrhosis? The sinusoidal pressure hypothesis and role of arterIALIZATION</td>
<td>MP-140</td>
<td>Sebastian Mueller</td>
</tr>
<tr>
<td>6</td>
<td>Directed differentiation of ips cells to hepatic stellate cells</td>
<td>MP-142</td>
<td>Mar Coll</td>
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<td>Screen</td>
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<tr>
<td>1</td>
<td>Integrin alpha 11 in regulation of myofibroblasts phenotype: implication for fibrotic diseases</td>
<td>MP-156</td>
<td>Ruchi Bansal</td>
</tr>
<tr>
<td>2</td>
<td>Simvastatin impedes the endotoxin-induced aggravation of hepatic microvascular dysfunction in two animal models of liver cirrhosis</td>
<td>MP-146</td>
<td>Dinesh M Tripathi</td>
</tr>
<tr>
<td>3</td>
<td>Accuracy of transient elastography in predicting histological fibrosis severity in treated autoimmune hepatitis</td>
<td>MP-148</td>
<td>Laura Harrison</td>
</tr>
<tr>
<td>4</td>
<td>Coffee consumption is protective of liver stiffness in the general population: the rotterdam study</td>
<td>MP-155</td>
<td>Juliana Fittipaldi</td>
</tr>
<tr>
<td>5</td>
<td>Alterations in cationic amino acid transporters and oxidative stress in the development of non-alcoholic steatohepatitis</td>
<td>MP-159</td>
<td>Laura Giuseppina Di Pasqua</td>
</tr>
<tr>
<td>6</td>
<td>Epithelial and mesenchymal molecular profiles in an experimental microsurgical model of cholestasis. Their relations to epithelial-mesenchymal transition and hepatic fibrosis</td>
<td>MP-162</td>
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INVITED SPEAKERS’ ABSTRACTS
Persistent or dysregulated IL-13 responses are key drivers of wound repair and fibrosis in multiple organ systems, identifying this cytokine as an important therapeutic target. Nevertheless, the mechanisms by which IL-13 inhibition leads to the amelioration of fibrosis have remained unclear. Because IFN-g exhibits potent anti-fibrotic activity, and IL-4Ra signaling antagonizes IFN-g effector function, compensatory increases in type 1 inflammation following IL-13/IL-4Ra blockade might contribute to the reduction in fibrosis. To investigate the potential influence of type-1 immunity, we developed IL-13-/-/IFN-g-/- double cytokine deficient mice and examined disease progression in models of type 2 driven fibrosis. As predicted, we showed that fibrosis in the lung and liver is highly dependent on IL-13 signaling. We also observed increased IFN-g production and inflammatory activity in the tissues of IL-13-deficient mice. Surprisingly however, a greater reduction in hepatic fibrosis was observed in IL-13/IFN-g double deficient mice following infection with the helminth parasite Schistosoma mansoni. The increased protection was associated with marked decreases in TGFb1, MMP12, and TIMP1 mRNA expression in the tissues, reduced inflammation, and decreased expression of important pro-inflammatory mediators like TNF-a. Experiments conducted with neutralizing monoclonal antibodies to IL-13 and IFN-g confirmed the findings with the genetically deficient mice. Together, these studies demonstrate that the reduction in fibrosis observed when IL-13 signaling is suppressed is not dependent on increased IFN-g activity. Instead, therapeutic strategies that block IL-13 and IFN-g activity simultaneously may confer greater protection from fibrosis by reducing the tissue-damaging type-1 inflammation that is seen when only IL-13 is targeted.

Disclosure of Interest: None Declared
IMPACT OF ETIOLOGY ON LIVER FIBROSIS

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Liver fibrosis is the hallmark of all chronic liver diseases. This unified pathway leads to cirrhosis and at this late stage, the etiology has little impact on the histological pattern. However, this is at the early phase of the disease that etiology strongly impact the pattern of fibrosis so that histology allows generally to identify the etiology that lay behind the fibrotic process. Indeed, the location of early fibrosis (periportal, perisinusoidal or zone 3), the type of fibrous septa and their location (porto-central, porto-portal or centro-central) are closely dependent of etiology. Finally, although cirrhosis is a single feature defined by annular fibrosis, there are also morphological patterns that may vary across etiologies. These different patterns can shade light onto the different mechanisms that drive fibrogenesis in these various conditions.

In chronic viral or autoimmune hepatitis, liver fibrosis develop and extent from portal tracts. Collagen fibers are deposited in a radiated organisation around portal tract and extend throughout the lobule. Portal and periportal inflammation drive fibrosis in this condition and there is a close association between periportal inflammation (interface hepatitis) and deposit of extracellular matrix along the periportal sinusoids. The formation of fibrous septa (bridging fibrosis between adjacent vascular structures) is closely related to the structure of the liver acinus. Bridging necrosis (bridge of necroinflammatory areas between portal tract and central vein) is an accelerator of the formation of fibrous septa between portal tract and zone 3 of the lobule. They are mostly seen in autoimmune hepatitis or Hepatitis B while they are uncommon in Hepatitis C. Convergence of fibrous septa and periportal fibrosis leads to annular fibrosis (cirrhosis) which is usually made of nodules of regular size.

Fibrosis in chronic biliary diseases (primary biliary cirrhosis, primary sclerosing cholangitis or even chronic bile duct obstruction) also start from the portal tract. Although inflammatory reaction might be significant, it is usually marginally involved in progression of fibrosis since interface hepatitis is usually absent. The characteristic pattern associated with biliary fibrosis is periportal ductular proliferation. Although ductular proliferation may be present in other chronic liver diseases, it is the major feature which is associated to fibrosis progression in biliary disease. The relationship between ductular proliferation and fibrogenesis is unclear although several studies suggest a possible role for epithelio-mesenchymal transition. One characteristic of biliary fibrosis is the extension from portal to adjacent portal tract with usual preservation of the zone 3, even in the advanced stage. Biliary cirrhosis is more irregular with usually a typical incomplete (jigsaw) or macronodular pattern.
Fibrosis in alcoholic and non-alcoholic liver diseases display a similar and characteristic pattern with early involvement of Zone 3 of the lobule. Cellular inflammation is usually mild, even in advanced fibrosis. A very specific pattern is perisinusoidal fibrosis, a characteristic chicken-wire deposit of collagen fibers along the sinusoidal wall. The formation of fibrous septa bridging central vein and portal tract is a later event drove both by the extension of zone 3 fibrosis and the development of periportal fibrosis. In ALD and NAFLD, perisinusoidal fibrosis may develop and extent anywhere in the lobule. A dense and diffuse perisinusoidal fibrosis is also very characteristic of Type 2 Diabetes. Perisinusoidal fibrosis may also differentiate NAFLD-related cirrhosis to other type of cirrhosis.

Vascular disease related to chronic central vein obstruction (such as Budd Chiari syndrome) or supra-hepatic obstruction leads also to a zone 3 fibrosis which is often associated with sinusoidal congestion. In this condition, fibrosis evolve to central to central bridging with, at the end, the typical feature of inverted cirrhosis that preserve the portal tract. The pathophysiological mechanisms that drive this fibrosis pattern might be related to both increase in zone 3 perisinusoidal pressure and sinusoidal microthrombosis related to blood stasis.

**Disclosure of Interest:** None Declared
IMPACT OF GENETICS ON LIVER FIBROSIS

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Hepatic fibrosis is a non-specific reaction to any chronic liver injury and is caused by exogenous factors such as viral hepatitis or alcohol, but family and epidemiological studies have also pointed to a role of genetic factors. Gene mutations can cause hereditary liver diseases and fibrosis. Examples represent hemochromatosis, Wilson disease, 1-antitrypsin deficiency, and progressive familial intrahepatic cholestasis (biliary fibrosis). Next-generation sequencing has proven to be helpful for the identification of novel disease genes and mutations in individual patients with fibrosis and cirrhosis of unknown aetiology. However, even in monogenic diseases the phenotypic spectrum and penetrance may differ between carriers of the same mutation, indicating that complex interaction between genes, genome and other factors underlie disease manifestation and fibrosis progression. In fact, each human genome harbours 2,000 variants associated with complex traits and about 30 mutations implicated in rare diseases. Common liver diseases are attributed to exogenous factors, in particular alcohol, over nutrition, and physical inactivity. Some of these patients show mild morphologic and functional alterations and are characterized by slow progression of liver fibrosis, whereas others develop pronounced fibrosis rapidly, culminating in cirrhosis and hepatocellular carcinoma. These differences in fibrosis progression persist when controlling for age, sex and exogenous factors in multivariate analysis, indicating that genetic factors play critical roles in modulating fibrogenesis. Genome-wide association studies have identified these genetic factors in experimental models and patients. The strongest and most robustly replicated genetic associations in both non-alcoholic and alcoholic fatty liver disease are with two genes: patatin-like phospholipase domain-containing 3 (PNPLA3) and transmembrane 6 superfamily member 2 (TM6SF2). The former encodes the triglyceride (TG) hydrolase adiponutrin, and TM6SF2 affects the secretion of TG-rich lipoproteins. The PNPLA3 variant is common worldwide, whereas the TM6SF2 mutation p.E167K confers lower population-attributable risk. A single mutation in these genes is neither sufficient to cause liver disease nor to predict the development of advanced fibrosis but the gene variants might serve to identify livers that are more or less “vulnerable” or “resilient” to fibrogenesis. In chronic hepatitis C virus infection, replication studies showed that PNPLA3, MERTK and TULP1 genotypes add to the risk of accelerated fibrosis progression rate. Detailed information on liver disease phenotypes and behaviour could serve to guide basic research as well as gene-stratified surveillance and therapy in clinical practice.

Disclosure of Interest: None Declared
IMPACT OF EPIGENETICS ON LIVER FIBROSIS

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Irrespective of the cause of chronic liver disease, only a minority of patients will develop severe fibrosis. The molecular basis for this variance is not known and we currently have no methodologies for predicting prognosis, discerning early stages of fibrosis or tracking the scarring as it progresses. Epigenetic processes play a prominent role in a number of complex diseases and most likely also mediate the effects of environmental factors including diet. This plasticity of epigenetic marks and molecules in response to environmental and genotypic influences may help explain inter-individual differences in speed of liver disease progression.

Epigenetic mechanisms comprise histone modifications, non-coding RNAs and DNA methylation. Combination of these mechanisms, in conjunction with transcription factors, instructs the expression of genes in cells, ultimately determining cellular phenotype. We have previously shown that differential DNA methylation of fibrosis modifier genes in the liver can stratify patients in terms of fibrosis severity. Latest studies show that same DNA methylation blueprint can be detected within the pool of cell-free genomic DNA found in human plasma, which can be used to stratify liver fibrosis severity in patients with NAFLD and ALD. These signatures can be traced back to the molecular pathology in fibrotic liver tissue, providing a biomarker of the underlying pathological process that may be able to track fibrosis by severity using a blood test.

Disclosure of Interest: None Declared
Recent evidence highlights the relevance of the crosstalk between gut and liver and its relevance for fibrosis progression. The gut microbiome promotes metabolism and digestion in symbiotic relationship with the host. Here different factors, e.g. alcohol or metabolic insults trigger changes in microbiome composition and consequently dysfunction of the intestinal barrier (“leaky gut”). Hence changes in gut microbiome and its homeostasis are associated with translocation of bacteria and/or bacterial products to the liver contributing to liver disease progression.

The integrity of the intestinal mucosa is determined by the function of distinct components: a protective mucin layer of defense on the intraluminal surface of the intestinal epithelium, tight junction proteins between intestinal epithelial cells, and the gut immune cells in the intestinal wall. Changes in this tightly regulated defense system contribute to increased gut permeability, which enhances translocation of bacterial endotoxins such as lipopolysaccharides (LPS) from the intestinal lumen into the portal circulation, thereby activating hepatic Kupffer cells via e.g. Toll-like receptor 4 (TLR4) to produce pro-inflammatory cytokines and chemokines. As a consequence, neutrophils and monocytes are attracted into the liver and play a critical role in liver disease activity by contributing to HSC activation and fibrosis initiation as well as progression.

In the actual presentation an update on the recent evidence will be summarized. This will include the impact of the immune system on gut microbiome composition, etiology-specific aspects on microbiome changes and its influence on Gut-Liver communication.

Disclosure of Interest: None Declared
Hepatic macrophages are central in the pathogenesis of chronic liver injury and have been proposed as potential targets in combatting fibrosis. Experimental studies in animal models as well as comprehensive studies from human samples revealed that hepatic macrophages are a remarkably heterogeneous population of immune cells that fulfill diverse functions in homeostasis, disease progression and regression from injury. These range from clearance of pathogens or cellular debris and maintenance of immunological tolerance in steady state conditions; central roles in initiating and perpetuating inflammation in response to injury; promoting liver fibrosis via activating hepatic stellate cells in chronic liver damage; and, finally, resolution of inflammation and fibrosis by degradation of extracellular matrix and release of anti-inflammatory cytokines. Cellular heterogeneity in the liver is partly explained by the origin of macrophages. Hepatic macrophages can either arise from circulating monocytes, which are recruited to the injured liver via chemokine signals, or from self-renewing embryo-derived local macrophages, termed Kupffer cells. Kupffer cells appear essential for sensing tissue injury and initiating inflammatory responses, while infiltrating Ly-6C+ monocyte-derived macrophages are linked to chronic inflammation and fibrogenesis. In addition, proliferation of local or recruited macrophages may possibly further contribute to their accumulation in injured liver. During fibrosis regression, monocyte-derived cells differentiate into Ly-6C (Ly6C, Gr1) low expressing ‘restorative’ macrophages and promote resolution from injury. Understanding the mechanisms that regulate hepatic macrophage heterogeneity may help to develop novel macrophage subset-targeted therapies for liver disease. Studies are ongoing to pharmacologically inhibit inflammatory monocyte influx into the liver, e.g., by blocking CCL2 or CCR2, or to augment the differentiation of restorative macrophages.

**Disclosure of Interest:** F. Tacke: Grant/Research Support: Noxxon, Tobira, Consultant/Advisor: Tobira, Galapagos, Boehringer, Intercept
INNATE LYMPHOID CELLS IN LIVER INFLAMMATION,
FIBROSIS AND REPAIR

Veronika Lukacs-Kornek*

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Apart from circulating immune cells, the liver accommodates multiple subsets of tissue resident innate lymphoid cells. Latter includes a diverse family of lymphocytes such as natural killer T (NKT) cells, natural killer (NK) cells and type 1 innate lymphoid cells (ILC1). Multiple studies have demonstrated that these cells significantly influence inflammatory processes in the liver. Although the tissue resident lymphoid compartment contains a diverse group of cells with differing immunological functions, increasing evidence suggest that they are collectively involved in the maintenance of tissue integrity. The fibrosis promoting and anti-fibrotic effects of the various innate cell subsets will be highlighted with special attention to their role in regeneration and restoration of liver tissue homeostasis.

Disclosure of Interest: None Declared
LYMPHOCYTE-ENDOTHELIAL INTERACTIONS: WHY ARE THEY IMPORTANT?

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Blood vessels within the liver present a unique environment through which immune cells can be recruited into tissue in response to infection or injury, or as part of normal immune homeostasis. There is also a well-established link between presence of key immune cell populations and fibrosis. It is clear that the timing of recruitment and the type of cells that enter the liver shapes the patterns of liver injury and repair. The nature of the inflammatory stimulus determines whether portal inflammation or lobular hepatitis is predominant and this is regulated by the differential expression of adhesion molecules and chemokines on vascular and sinusoidal endothelium which act together to compartmentalize infiltrating lymphocytes. Expression of proadhesive molecules on structures such as biliary epithelium and activated myofibroblasts also helps localize and retain immune cells at key anatomical sites. In all cases, initial recruitment of cells from the bloodstream and into tissue is dependent upon interactions between blood cells and hepatic endothelium. Vessels in the normal liver express low levels of adhesion molecules and chemokines, which are induced and/or upregulated in response to injury and liver inflammation. These include CC- and CXC-chemokines and classical adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) as well as the atypical adhesion receptor and amine oxidase, vascular adhesion protein-1 (VAP-1) amongst others. Emerging data also highlights how additional players such as platelets can influence immune cell recruitment and activation during hepatic injury. An understanding of the signals that regulate the processes of lymphocyte recruitment confers the ability to selectively influence the process therapeutically, and thus targeting inflammation has important consequences for developing and modifying hepatic fibrosis.

Disclosure of Interest: None Declared
FRAMING THE INFLAMMASOME

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The innate immune responses to tissue injury caused by pathogens, cellular stress or environmental insults initiated by the cytosolic nucleotide-binding domain and leucin-rich repeat receptors (NLRs), retinoic acid-inducible gene (RIG) like helicase (RLH) receptors and absent in melanoma (AIM)-like receptors.

Some NLRs form inflammasomes, which act as scaffolding proteins within a complex of caspase-1 and in some cases an adaptor protein. To date, the NLR family comprises 14 NLR genes identified in human and 20 in mouse. Within the liver inflammasomes are expressed in both parenchymal and non-parenchymal cells and serve as key regulators of inflammation and cell fate. Among all inflammasomes, NLRP3 has been shown to be most important in the response to sterile inflammation. NLRP3 assembles a complex comprised of the adaptor protein apoptosis associated speck like protein (ASC) and the serine protease caspase-1. The Nlrp3 inflammasome responds to cellular danger signals by activating caspase-1, releasing IL-1 and IL-18, as well as initiating a novel pathway triggering programmed cell death termed pyroptosis. Activation of the NLRP3 inflammasome in mice results in severe liver inflammation, fibrosis and hepatocyte pyroptotic cell death. In murine models of alcoholic as well as non-alcoholic steatohepatitis (ASH and NASH), NLRP3 activation is required for the fibrotic response suggesting that targeting this complex may be a rational strategy to block or reverse the development of fibrotic liver disease. Among the various cytokines participating in chronic hepatic inflammation IL-1β plays a special role. IL-1β was found to promote hepatic stellate cell proliferation, activation, and trans-differentiation into a myofibroblast phenotype. Therapeutic strategies to reduce Nlrp3 inflammasome activity encompass compounds that affect Nlrp3 inflammasome assembly and activation, caspase 1 activation, and IL-1 and IL-18 pathways.

Disclosure of Interest: None Declared
In patients with chronic liver disease, fibrosis is favoured by the presence of obesity or overweight, which are also relevant risk factors for the progression of non-alcoholic steatohepatitis. Accumulating evidence indicates the modulation of the fibrogenic process by adipokines, a group of cytokines secreted primarily by adipose tissue [1]. Most of the available data are related to the role of leptin and adiponectin, which favour and limit the fibrogenic process, respectively.

Leptin has been shown to mediate profibrogenic effects in the liver, while the effects of adiponectin are mostly anti-fibrogenic and anti-inflammatory [1]. Recently, leptin was found to upregulate the expression of CD14 in Kupffer cells, resulting in enhanced response to low-dose endotoxin and accelerated progression of NASH [2]. These data demonstrate a cross-talk between leptin and innate immunity via toll-like receptor (TLR)-4. In mice with diet-induced obesity treated with CCl4, leptin was found to play a key role in peroxynitrite-mediated oxidative stress in macrophages and Kupffer cells [3]. Moreover, circulating and hepatic levels of leptin are increased by CYP2E1, an isoform of liver CYP450 that stimulates NASH progression generating reactive oxygen species and free radical metabolites, highlighting the tight connection between leptin and oxidative stress.

The protective role of adiponectin on fibrosis occurs via several mechanisms, and recently an adiponectin-induced increase in the expression of the co-receptor Bambi has been shown to limit the effects of transforming growth factor (TGF)-beta1 on the expression of fibrogenic factors by hepatocytes [4]. In addition, adiponectin inhibits hepatic fibrosis through binding of suppressor of cytokines signaling-3 (SOCS-3) to the long form of leptin receptor (Ob-Rb) and stimulation of expression and activity the protein tyrosine phosphatase 1B [5]. Adiponectin’s action has been recently linked to bile acid metabolism. In obese patients receiving bariatric surgery, bile acid (BA) synthesis and levels increase with severity of NAFLD, and expression of genes involved in hepatic BA uptake and biosynthesis (NTCP and CYP7A1) is augmented, indicating failure to activate small heterodimer partner (SHP) upon farnesoid X receptor (FXR) stimulation by increasing BA concentrations. Adiponectin was inversely correlated with serum BAs and hepatocellular death and a potential effect of adiponectin on genes related to BA homeostasis has been proposed [6].
Other adipokines could possibly participate in the regulation of hepatic fibrosis. Using a model of bile duct ligation, Dong et al. demonstrated that resistin treatment directly and indirectly modulates HSC behavior towards a more pro-fibrogenic phenotype [7].

References

Disclosure of Interest: None Declared
ANGIOGENESIS AND LIVER FIBROSIS

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Angiogenesis defines the growth and proliferation of blood vessels. Angiogenesis has been linked with fibrogenesis in a number of organ systems including the liver. While vascular endothelial growth factor is the canonical angiogenic molecule most studied, a number of other angiogenic molecules have similar or distinct effects. However, the causal relationship between angiogenesis and fibrosis is not fully established. Furthermore, a number of complexities are emerging since angiogenesis may also be required for fibrosis resolution, thereby making therapeutic intervention difficult. Angiogenesis in liver and associated vascular beds has also been linked with portal hypertension. A number of new therapies are emerging for fibrosis and portal hypertension which affect angiogenesis including the statin class of drugs. The effects of statins on angiogenesis are complex and variable. This talk will cover our current understanding of angiogenic factors in fibrosis (and portal hypertension as time permits). Additionally, new data will be shown relating to the vascular protein synectin and its role in hepatic fibrogenesis. If time permits, new data sets will also be shown relating to another protein, TANGO1 and its role in hepatic fibrosis.

Disclosure of Interest: None Declared
IMMUNOREGULATION OF LIVER FIBROSIS

Sophie Lotersztajn

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Sustained hepatic inflammation resulting from parenchymal liver injury is a major driving force of both fibrosis progression and fibrosis resolution. Therefore, there is a growing interest in the identification of the mechanisms that control liver inflammation in the context of liver fibrosis. Convergent data demonstrate that the hepatic innate and adaptive immune system play a key role in the initiation, perpetuation and maintenance of the inflammatory response, with major deleterious impact on hepatocyte lesions and fibrosis. Recent studies have also highlighted the contribution of innate lymphoid cells in the fibrogenic process. We will discuss the latest advances on the role of innate, adaptive immune cells and innate lymphoid cells in the control of liver fibrosis. We will also summarize our recent findings on the identification of anti-inflammatory targets and pathways that control Kupffer cell phenotype in experimental models of fibrosis, in particular components of the endocannabinoid system and the autophagy pathway. Finally, we will present some data regarding the role of non-conventional T cells such as MAIT cells in the control of fibrosis progression.

Disclosure of Interest: None Declared
ACTIVATION OF PROGENITOR CELLS IN LIVER FIBROSIS

Pau Sancho-Bru*

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The healthy liver is a slow turnover tissue. However, after injury, the liver possesses an extraordinary regenerative capacity, being able to rapidly restore its mass even after a substantial mass loss such as in partial hepatectomy or during chronic liver injury. After injury, hepatocytes and cholangiocytes, the two epithelial cell types in the liver, as well as non-parenchymal cells proliferate to replace injured cells and restore the liver mass. The liver has been proposed to have facultative stem cells or progenitor cells. Under some circumstances, when hepatocytes lose their replicative capacity and are no longer able to sustain hepatocyte regeneration, liver progenitor cells are supposed to start proliferating thus giving rise to what is known as ductular reaction. Although liver progenitor cells have been isolated and proved to have bipotential capacity in vitro, its contribution to newly generated hepatocytes in vivo is still debated, and it is not clear whether, and to what extent, they contribute to liver regeneration. Another controversial issue in the field of liver progenitor cells is their origin. While stem/progenitor cells have been suggested to exist in the canals of Hering or in peribiliary glands, recent data suggest that hepatocytes have an important plasticity and in the context of liver injury, dedifferentiation of mature hepatocytes may be the origin of an immature cell population with hepatobiliary phenotype. Finally, in this context, important differences between animal models and human diseases may exist, and different mechanisms of regeneration, control of cell division and differentiation may be taking place. What is the origin of ductular reaction and liver progenitor cells? What are the cues that induce their expansion? What is their function and role in chronic liver disease? What is the cross-talk between progenitor cells and non-parenchymal cells in the liver? All these are open yet important questions with no clear answer.

Irrespectively of the origin of ductular reaction cells, it is clear that they are present in most chronic liver diseases, and in some particular conditions such as in alcoholic hepatitis, ductular reaction cells represent a substantial population in the liver. The expansion and phenotype of ductular reaction cells depends on the type of liver injury, but both in humans and in mouse models, ductular reaction correlates with disease severity, and is associated with hepatocyte injury and senescence. In human liver disease, ductular reaction and expression of liver progenitor cell markers correlate with fibrosis progression, and the type of extracellular matrix influence their proliferation, phenotype and differentiation capacity. Moreover, recent studies not only suggest that ductular reaction cells are not an inert cell population, but also that they may be an important player in the pathogenesis of chronic liver disease. The lecture “Activation of progenitor cells in liver fibrosis” will review the
importance of extracellular matrix composition in liver progenitor cells proliferation and differentiation. Moreover, it will discuss the latest findings regarding liver progenitor cell phenotype in chronic liver disease and its potential role in liver inflammation and progression of liver fibrosis.

**Disclosure of Interest:** None Declared
CIRRHOSIS OR CIRRHOSES?

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The term “cirrhosis” identifies an advanced phase of chronic liver diseases (CLD) that per se is neither morphologically or clinically an end stage, particularly with the prospective of novel treatments able to stabilise or even reduce tissue fibrosis. Regardless, patients with cirrhosis are classified along with the appearance of clinical manifestations typical of decompensated cirrhosis with little or no ability to predict these often life threatening events (so called ‘expectant’ algorithm). In addition, it is increasingly clear that different CLD are characterised by different predominant pro-fibrogenic mechanisms and, while cirrhosis is the common result of progressive fibrogenesis, there are distinct patterns of fibrosis development in different and even within the same CLD. These aetiology-related patterns are linked to the relative prevalence of different pro-fibrogenic mechanisms, such as the activation of a chronic wound healing reaction, oxidative stress and derangement of the epithelial-stromal equilibrium around bile ducts. The knowledge of these aspects of the pathophysiology of CLD leads to the awareness that a correct interpretation of the development of cirrhosis should take into consideration the correlation between time of progression of liver disease, the aetiological agents, the dynamics of the necro-inflammatory infiltrate, the distribution of fibrosis and the onset and progression of portal hypertension (PH), depending on the aetiological agent.

Considering the immediate prospective of treating efficiently a very large number of cirrhotic patients with the new IFN-free regimens, there is a concrete possibility that a significant number of patients with compensated advanced chronic liver disease due to HCV will achieve sustained viral response (SVR) and possibly regression of liver tissue fibrosis. However, the available data obtained over the past decade in cirrhotic patients who had achieved SVR, indicate that in patients with clinically significant and severe PH, HCV clearance does not induce a significant reduction of PH and that cirrhosis, once advanced may progress to decompensation even in absence on HCV replication. In biological terms, this can be explained by the relative autonomy acquired by the fibrogenic process beyond a certain level of development over decades characterised by chronic fibro-inflammation and neo-angiogenesis. In particular, it is conceivable that, at this stage of the disease, two major determinants may condition further clinical progression independently of the reduction of hepatocellular necrosis and inflammation induced by SVR. The first is represented by the remarkable hyperplasia of different types of activated fibrogenic myofibroblasts which is associated by a strong activation of anti-apoptotic pathways in these cells. The second is due to the extensive changes in hepatic angioarchitecture consequent to neoangiogenesis
and to the contraction of scar tissue leading to elevated tissue tension which are only minimally affected by the reduction of necro-inflammation following SVR.

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LIVER FIBROSIS AND PORTAL HYPERTENSION

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In patients with chronic liver disease, portal hypertension and progressive fibrosis are concomitant pathological processes interacting with each-other and leading to severe complications. Interruption of the liver injury either by treating the initial liver injury and addressing the perpetuating risk factors will improve both fibrosis and prevent or ameliorate portal hypertension. Currently, after the successful cure of viral hepatitis, lifestyle-related liver damage due to chronic alcoholism or morbid obesity will remain the main factor leading to liver fibrosis and portal hypertension. Even though, the basic pathogenetic mechanisms of development of fibrosis and portal hypertension are similar. Especially RhoA/Rho-kinase pathway is crucially involved in the pathogenetic processes inside and outside the liver. The modulation of these targets has been evaluated in different animal models. Also, some well-established drugs, which are used in humans for other indications (for example, statins), are promising if applied early and concomitantly to standard therapy. In the future, more cell-specific targeting and personalized strategies must be considered to avoid progression of disease and complications.

Disclosure of Interest: None Declared
CIRRHOSIS AS A SYSTEMIC DISEASE

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The anatomical and the functional position of the liver situated between the gut and the systemic circulation provides it the incredible ability to regulate the exposure of the organs to products coming from the gut and therefore when the liver fails in cirrhosis effects are observed in almost all organs. The liver is also the largest immunological organ containing the largest concentration of resident macrophages, the Kupffer cells. It is not surprising therefore, that failure of the liver is associated with unregulated inflammatory responses, inflammation and immune failure. Finally, the systemic metabolome is controlled by a close harmonious relationship between the microbiome and liver metabolic function. Therefore, liver failure is characterized by the failure of metabolic regulation, the effects of which are felt by almost all organ systems. With this background it is not surprising that functional syndromes such as hepatorenal syndrome, hepatic encephalopathy, hepatopulmonary syndrome, cirrhotic cardiomyopathy and hepatoadrenal syndrome are characterized by functional failure of these organs, with little or no observable evidence of organ injury and complete resolution with liver transplantation. Even in the face of severe acute liver injury, the outcome of patients is dictated by the occurrence of hepatic encephalopathy. The systemic nature of cirrhosis is really best illustrated and encapsulated in the newly defined syndrome, acute on chronic liver failure; a syndrome characterized by increased bacterial translocation, inflammation, altered host response to injury, immune failure and multiorgan failure. The number of organs failing is directly related to the risk of death. In conclusion, cirrhosis is truly a systemic disease with abnormalities in each organ system.

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CIRRHOSIS AS A PRE-NEOPLASTIC CONDITION

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Hepatocellular carcinoma (HCC) is one of the leading causes of death by cancer worldwide. It is mainly developed on cirrhosis due to chronic hepatitis B and C, metabolic and alcoholic liver diseases in western countries. Recent advances in molecular classification and dissection of genetic and epigenetic drivers have increased our knowledge of the molecular diversity of malignant liver tumours. Using genomic approaches, we identified several new oncogenes and tumour suppressor genes and we described a molecular classification of hepatocellular carcinoma. Recently, using sequencing, we identified TERT promoter mutations activating telomerase as the most important mechanism of malignant transformation of cirrhotic nodules in carcinoma. We also found new mutational signatures in HCC as the result of exposure to specific genotoxic agents. Finally, next generation sequencing was particularly fruitful to identify new drug targets in hepatocellular carcinoma and these finding open new avenues to develop genome based clinical trials.

Disclosure of Interest: None Declared
FIBROSIS AND CIRRHOSIS REGRESSION: 
CLINICAL IMPLICATIONS (THE POST HCV SCENARIO)

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The recent advances achieved in the treatment of HCV by the development of new
direct-acting antiviral agents (DAAs) allow to treat, with a very high likelihood to obtain
a sustained virological response, a broad spectrum of patients, including those with
advanced liver disease.

Data from large trials comparing pre- and post-treatment liver biopsies demonstrate
improvements in inflammation as well as fibrosis after SVR. However, albeit the histological
semiquantitative and quantitative evaluation of liver fibrosis remain the most reliable
parameters to assess short-term benefit of viral clearance, several methodologies have been
proposed for the noninvasive monitoring of liver fibrosis in chronic HCV hepatitis. Indeed,
with the introduction of IFN-free regimens for HCV, monitoring of fibrosis regression
following SVR will be largely performed with transient elastography (TE) even if we still
not have reliable data on the performance of this technique in assessing the true reduction
of liver fibrosis since its result may be influenced by many factors like the reduction of
necroinflammation, changes.

In many cohorts of patients with established HCV cirrhosis, the achievement of SVR was
related with a significant lower risk to develop progression of portal hypertension, liver
decompensation, hepatocellular carcinoma and liver related death.

Also the last Baveno Consensus changed the recommendation for surveillance of
esophageal varices according to the clearance of the aetiological factor, suggesting to
repeat surveillance endoscopy at longer intervals in patients with SVR.

Nevertheless, in all studies analyzing the long term benefit of SVR in patients with cirrhosis,
ea small subset of patients experienced a progress to cirrhosis despite SVR, developing
EV progression and or LD and HCC. The potential mechanisms involved in those cases
of disease progression despite SVR are not still explained and therefore the outcome of
single patient is not clearly predictable. For this reason clinical and ultrasound surveillance
should be performed also in patients with HCV clearance.

We should also take into account that by the use of DAAs we are “curing” the HCV
infection in a considerable number of patients with clinical significant portal hypertension
and/or with previous or concomitant signs of liver decompensation. Only preliminary data
suggest that in a part of those patients, SVR may be able to reduce MELD score or HVPG
but further studies are necessary to better focus this issue and assess if exist “a point of no
return” for cirrhosis regression.

Disclosure of Interest: None Declared
THE CELLULAR AND MOLECULAR COMPONENTS OF FIBROSIS REGRESSION

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A multitude of animal models and large-scale clinical trials of antiviral therapies in chronic hepatitis B and C have demonstrated unequivocally that liver fibrosis and even cirrhosis are potentially reversible if the underlying cause can be successfully eliminated. However, in a significant proportion of patients disease-specific treatment may not result in fibrosis regression, decrease in portal hypertension, or a significant reduction of the risk for hepatocellular carcinoma development. An increased understanding of the mechanisms that characterize matrix remodeling and architectural repair during fibrosis regression, as well as factors such as collagen crosslinking that limit these processes, is informing therapeutic strategies to induce or accelerate regression as well as novel diagnostic tools. Recent seminal observations in rodent models have determined that in resolving liver fibrosis a significant proportion of fibrogenic hepatic stellate cell-myofibroblasts can revert to a near quiescent phenotype. Additionally, hepatic macrophages derived from inflammatory monocytes may contribute to fibrogenesis and also fibrosis resolution through an in situ phenotypic switch mediated by phagocytosis, while other discrete inflammatory cell subsets may also promote regression. Macrophage-derived vascular endothelial growth factor and angiogenesis within the hepatic scar have recently emerged as key processes in the resolution of hepatic fibrosis in mice. An increasing number of therapeutic approaches are now being translated from the bench to the clinic including the modulation of hepatic stellate cell activation and fibrogenic behaviour, manipulation of macrophage kinetics and phenotype, and autologous cell infusion therapies. In parallel, novel non-invasive diagnostic tests such as serum and imaging biomarkers responsive to extracellular matrix degradation, portal hypertension and liver regeneration are being developed to evaluate the clinical efficacy of antifibrotic and pro-resolution interventions.

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HOW TO ASSESS REGRESSION: PATHOLOGY?

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Introduction

Fibrosis regression is a separate issue to cirrhosis regression. Liver fibrosis and architectural changes are related, but they are not the same thing exactly. Up to now the mechanisms and morphology of progressive disease have been studied much more than regression. Uncritical application of our ideas about disease progression to regression is likely to be misleading.

Histological assessment of liver fibrosis

Liver fibrosis assessment is best accomplished by collagen proportionate area (CPA) measurement. There is no reason to suppose that fibrosis measurement methodology should be any different for regressing vs progressive disease. Quantitative studies of fibrosis regression are scarce. Most of the evidence for fibrosis regression consists of reduction of stage scores (which conflate fibrosis with liver disease stage) after treatment for viral hepatitis (Ellis EL and Mann DA. J Hepatol 56,1171; 2012).

Cirrhosis Regression

Stage score reduction in cirrhotics after treatment suggest cirrhosis can be reversed (Serpaggi J et al. Hum Pathol 37,1519;2006). A critical reappraisal of cirrhosis regression is needed, taking into account the abnormal vascular aspects of cirrhosis. Reliable identification of vascular shunts in cirrhosis regression remains beyond the reach of traditional histopathology. While CPA has been shown to be a useful surrogate for HVPG in progressing disease, this relationship has not yet been studied in liver disease regression.

Vascular aspects

The only study of (HCV) advanced stage liver disease with HVPG information and a post treatment biopsy showed ~4 mm Hg HVPG reduction mainly in SVR patients. Fibrosis (stage) reduction and HVPG reduction were not related, while HVPG decrease was greater in patients whose inflammation grade decreased (Rincon D et al. Am J Gastroenterol. 101,2269;2006).

Morphology

It is thought that highly fibrous micronodular cirrhosis, through a process of parenchymal regeneration, nodular enlargement, fibrosis reduction, and architectural remodelling becomes a less fibrous macronodular cirrhosis; then coalescence of nodules, attenuation of septa, and partial restoration of lobular structure results in an “incomplete septal cirrhosis” (ISC) appearance. Thus an ISC stage occurs in both the advance towards and the retreat from cirrhosis. Features (“hepatic repair complex”) suggestive of regressing cirrhosis (as it becomes ISC) have been proposed. These include perforated delicate septa, isolated collagen fibers, and aberrant parenchymal veins/portal adhesions (Wanless IR et al. Arch Pathol Lab Med 124,1599;2000), but more hepatic repair complex features were present in patients without SVR compared to SVR cases in liver biopsies after treatment.
for HCV (Pattullo V et al. Histopathology 61,473; 2012). Which histological features will indicate regression reliably is not yet known. Just as the pattern of disease progression is aetiology specific, so the features of regression are likely to be affected by aetiology also.

Restoration of lobular structure Biopsies from 38 HCV patients with cirrhosis, 5y after SVR were studied by D’Ambrosio R et al. (Hepatol 56,532;2012). Restoration of lobular metabolic zonation (CYP2E1 and glutamine synthetase immunostaining) was shown in the posttreatment regressed cirrhotic biopsies. Cirrhotic sinusoidal capillarisation reversal (CD34) was not seen.

Irreversible cirrhotic parenchymal changes “You can always come back, but you can’t come back all the way” (Dylan B. “Mississippi” 1997). There could be essentially irreversible cirrhotic parenchymal changes. In rats with CCl4 cirrhosis Di Vinicius I et al. (Pathol Res Pract 201;449;2005) found that after discontinuation of CCl4, an ISC appearance persisted until the end of the observation period (9 mths). Doratiotto S et al. (Histochem Cell Biol 135,581;2011) showed the repopulation of rat liver with normal hepatocytes resulted in a normal lobular architecture, while repopulation using cirrhotic hepatocytes resulted in nodular parenchyma. Other observations indicate HCC risk persists after cirrhosis regression.

Conclusion
Reduction of liver fibrosis is not unusual when the causative agent can be eliminated. Reversal of the architectural and vascular disruption of cirrhosis is a different matter. A better understanding is needed of the histopathological features of cirrhosis regression. In advanced stage liver disease, as antifibrotic agents come to clinical trial and as effective antivirals are used, if liver biopsies are to be done, if possible, we should choose transjugular biopsies with HVPG and CPA measurements to establish portal hypertension changes alongside fibrosis quantification and cirrhosis regression histopathology. Thereby we could achieve a broader experience of currently obscure important histological features of cirrhosis regression and their relationships with portal hypertension.

Disclosure of Interest: None Declared
Non-invasive tests have emerged over the past decade challenging liver biopsy for staging liver fibrosis. They rely on two different but complementary approaches (a “biological” approach based on the dosage of serum markers; a “physical” approach based on the measurement of liver stiffness using ultrasound-based elastography techniques) and are now widely used as first line tools for prioritising patients with viral hepatitis for antiviral treatment. The potential use of non-invasive tests for monitoring fibrosis progression and regression is mostly applicable to HCV as most studies published so far are in this field. There are two important methodological issues that should kept in mind when dealing with fibrosis regression or reversal. First, cirrhosis is a morphologic definition not allowing to distinguish between a fibrogenic process that is still in progress but potentially reversible, from a more advanced stage of the liver disease that becomes irreversible. To date, there is still no clear-cut demonstration whether the benefit of sustained virological response (SVR) on hard end points, such as reduction of liver-related death, need for transplant and hepatocellular carcinoma development, are equally reduced irrespective of the stage of HCV cirrhosis. Second, when thinking to use non-invasive tests, one should keep in mind that all of them were designed for the assessment of fibrosis progression and not for regression. For instance, in patients with SVR a significant decrease of liver stiffness is detected only after 1.5-3 years of follow-up and liver stiffness assessment earlier than 6 months after the end of therapy does not appear to reflect a regression of tissue fibrosis but rather a decrease in the extent of necro-inflammation. In the only study available so far in 33 patients with cirrhosis and SVR with pre- and post- treatment liver biopsies, transient elastography sensitivity was too low (61%) to be utilized clinically as evidence of cirrhosis regression. Further studies are needed to define more precisely the role of non-invasive methods in monitoring fibrosis regression.

Disclosure of Interest: None Declared
MOLECULAR MAGNETIC RESONANCE IMAGING OF LIVER FIBROSIS IN ANIMAL MODELS

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Non-invasive methods to detect and stage hepatic fibrosis and inflammation remain imperfect. Magnetic resonance imaging (MRI) offers high resolution, whole organ coverage of the liver with excellent fat and iron quantification. MR elastography allows measurement of liver stiffness. Molecular MRI can yield additional information on fibrosis and inflammation that is synergistic with conventional MRI readouts resulting in a comprehensive examination.

The aims of this work are to evaluate molecular MR probes targeted to collagen, fibrin, and oxidized collagen as imaging biomarkers of fibrosis, inflammation, and fibrogenesis in different rodent models of disease, and to compare the imaging readouts with conventional MRI, MR elastography, and ex vivo histological and biochemical methods.

Probes were evaluated in mouse carbon tetrachloride model and mouse choline deficient high fat diet as well as the rat diethylnitrosamine model and the rat bile duct ligation model. Animals were imaged at different stages of fibrosis progression. Molecular probes were EP-3533, a type I collagen specific probe, EP-2104R, a fibrin-specific probe, and Gd-Hyd, a probe targeting oxidized collagen. MR imaging included conventional relaxation time measurements, MR elastography, and molecular MRI. Following imaging, livers were analyzed by Sirius Red staining with quantification of collagen proportional area, Ishak scoring, and hydroxyproline analysis.

Collagen molecular MR imaging was found to accurately detect and stage fibrosis in all the animal models used. The molecular MR readout was two orders of magnitude more sensitive for staging fibrosis than conventional MR measures. Collagen molecular MRI was found to be complementary to MR elastography and the two methods could be combined to increase diagnostic accuracy of staging disease. Fibrin molecular imaging is a promising method to assess hepatic inflammation using extravascular fibrin as a surrogate. Molecular imaging of oxidized collagen represents a new technique to assess active fibrogenesis and to monitor treatment response.

Molecular MR methods are additive and complementary to existing MR approaches to stage liver fibrosis and to monitor treatment response. Molecular probes have been validated in rodent models of disease and clinical translation is warranted.

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NEW IN VITRO MODELS TO INVESTIGATE FIBROSIS – 3D VS 2D

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In the past 4-5 decades various approaches have been successfully employed to isolate hepatic cells. Separating and culturing a specific hepatic cell from the total hepatic cell suspension allows to study, in a much defined way, primary molecular signalling pathways associated with fibrogenesis or liver diseases in general. Despite major improvements in refining the isolation procedures and culture conditions in two-dimensional systems (2D), no efficient anti-fibrogenic therapy has been developed yet. Many of the promising compounds have failed when translated from 2D culture systems into in vivo models owing to the lack of the specific liver microenvironment.

A variety of cell culture models exist with different complexity. The most described and used model is the single mono-layer culture of hepatic cells isolated from healthy or diseased livers. Phenotypical differences, thus changes in cell behaviour, can be found depending upon culturing the cells on plastic, on collagen or cultured on specific extracellular matrix substrates. Co-culture is one way to investigate the intercellular communication between different hepatic cell types. Many examples have demonstrated a strong communication between hepatic cells such as Hepatic Stellate Cells with Kupffer, hepatocytes, liver sinusoidal endothelial cells and cancer cell lines. For example, one can investigate the paracrine interactions and measure the stimulation/reaction of a cell type when it is brought in contact with the secreted cytokines or growth factors produced by the other cell type i.e. non-contact co-cultures by using culture trans-well inserts or the conditioned medium. On the other hand, one can investigate the contact-dependent effects by culturing 2 cell types as one monolayer.

Notwithstanding all observations in 2D single and co-cultures these current in vitro models are still limited. It is becoming clear that the recreation of the liver microenvironment with cell-matrix interactions, cell-cell adhesion is essential in liver studies. Indeed, although the traditional 2D cell culture systems are proven to be valid in investigating possible mechanisms of cell behaviour and screening for drugs to some extent, many previous investigations revealed a lack in translation towards animal models and, more importantly, into clinical studies. Therefore, the development of a well-defined three-dimensional (3D) in vitro model, which mimics ECM structures as found in vivo, has gained strong interest. Indeed, matrix mechanics is a key parameter in regulating a range of cell behaviours such as cell proliferation, cell migration and ECM production. In fact, initial experiments
performed on matrigel or 3D type I collagen gels moved towards spheroid-based 3D culture. One can also create a chip with a cascade design in which the culture medium flows from one specific cell culture towards the 3D-spheroids containing another hepatic cell type. Many other 3D models such as 3D synthetic scaffolds, have demonstrated beneficial effects in maintaining specific cell types without dedifferentiation properties. Very critical for promoting liver cell-specific functions in vitro to co-culture hepatocytes with non-parenchymal cells either in direct contact or via paracrine stimulation in these systems. Thus, major efforts have been made to mimic the in vivo hepatic cell and hepatic cell-ECM interactions which may more closely mimic the physiologic state. Precision-cut liver slices (PCLS), or whole tissue cores, is an example of how a 3D in vitro model can be used to study the hepatic cell in a system that very closely reflects the in vivo situation with maintaining the intact hepatic architecture and cellular heterogeneity. By using this 3D in vitro model, behaviour of HSCs, endothelial cells, Kupffer cells, biliary epithelial cells and portal fibroblasts has been investigated.

The absence of excellent and rapid 3D in vitro screening assays has slowed or arrested further investigation of possible lead compounds before they can be tested in animal models and/or phase I clinical trials. During the last decade, much effort has been made to develop ECM scaffolds by using rodent tissues such as whole rat kidney, gastrointestinal tract as well as rat liver tissue. A recent study has demonstrated the use of human liver tissue as 3D bioscaffold. Basically, a decellularization process removes the cellular material and this generates a native ECM scaffold, which remains highly preserved. In this context, the repopulation of the scaffold with different cell types represents a highly dynamic in vitro culture system, which reflects more realistically the hepatic 3D microenvironment. Optimization and refining in vitro models which reproduce the liver microenvironment will lead to new objectives and to a possible new era in the search for antifibrogenic compounds.

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CRITICAL DISCUSSION OF MOUSE MODELS FOR LIVER FIBROSIS

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Much of our understanding of liver fibrosis has been gained using animal models. With the advent of transgenic mice, most studies on hepatic stellate cells and liver fibrogenesis have switched from rat to mouse models, allowing to study the contribution of specific pathways to fibrosis development or resolution. Moreover, rodent models of liver fibrosis are increasingly used to test novel antifibrotics in preclinical settings. Here, we will critically review mouse models of liver fibrosis, discuss key advantages and disadvantages of specific models and highlight disease-specific models such as NASH-induced liver fibrosis, alcohol-induced liver fibrosis, biliary liver fibrosis and virus-induced liver fibrosis. In addition, we will also review areas that require further optimization. Most importantly, we still lack approved anti-fibrotics that can halt or reverse fibrosis in patients despite numerous mouse studies in which genetic or pharmacologic inactivation of specific pathways has decreased fibrosis. This failure may be in part due to the lack of standardized preclinical models that incorporate key aspect of human liver fibrosis (causative agent, similar pathophysiology and omis/gene expression, similar modes of cell death and similar speed of fibrosis development) allowing to conduct preclinical studies in a realistic “mouse hospital” setting.

Disclosure of Interest: None Declared
TARGETING FIBROSIS IN VIVO

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Fibrogenesis in the liver is dynamic, with clinical and experimental evidence that even advanced fibrosis has the potential to undergo regression. There is therefore a strong rationale for targeting hepatic stellate cells (HSC), the major cellular drivers of liver fibrosis. The challenge is to learn how to manipulate HSC in vivo in a selective manner so as to more specifically target the fibrogenic process and limit effects on other cell types and mechanisms that are essential for liver homeostasis and function. Data will be presented to illustrate two potential strategies: (1) the discovery and exploitation of molecular mechanisms that are selective or unique to HSC and (2) the development of technologies for selectively targeting therapeutic molecules to HSC in vivo. Examples will be presented from recent work in the Mann laboratory as well as from the wider field.

Disclosure of Interest: None Declared
CLINICAL GRADE EXPANDED LIVER DERIVED STEM CELLS FOR INFLAMMATION & FIBROSIS

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Cell therapy using mature or stem liver derived cells has entered the clinical field as a new approach to treat congenital and acquired liver diseases. Adult Derived Human Liver Mesenchymal stem /progenitor Cells (ADHLSCs) are obtained after primary culture of the parenchymal fraction of collagenase digested livers. After large scale in vitro expansion, ADHLSCs are recovered and cryopreserved for clinical use to treat liver defects. This cell population expresses markers and secretes cytokines displaying immuno-modulatory, regenerative and anti-fibrotic properties. ADHLSCs are able to differentiate into hepatocytic cells both in vitro (after incubation with specific growth factors and cytokines) and in vivo (after transplantation into animal models and in clinical studies). Clinical safety with ADHLSCs (Hepastem®, Promethera Biosciences, Mt St Guibert, Belgium) has been demonstrated in a phase I trial including 20 infants and children.

End stage liver diseases are the consequence of a progressive interrelated processes including chronic inflammation, fibrosis and cirrhosis. Treatment with ADHLSCs aims now to address liver fibro-inflammatory disease, such as AoCLF and NASH. Indeed, beside potential tissue replacement, ADHLSCs are a multi-target medicinal product including inhibition of T cell proliferation, stellate cell activation, antigen presenting cell maturation, as well as inhibition of TNFα and collagen secretion. ADHLSC mechanisms of action include HLA-G, HGF, βFGF IL6, MMP1&2, IDO, PGE2, DLL4. Initial exposure to the innate immune system triggers immunomodulatory competencies of MSCs to evade alloimmune rejection. Applying these multi-properties in proper clinical trials is opening new avenues for cell-based therapy to address unmet medical diseases.

Disclosure of Interest: None Declared
HOW TO TRANSLATE BASIC CONCEPTS TO TRIAL ENDPOINTS

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Prevention or reversal of cirrhosis has become a prime endpoint in trials of patients with chronic liver disease. Significant progress has been made in understanding the mechanisms underlying the development of liver fibrosis. This includes its dynamic nature, the plasticity of all liver cell populations, especially immune cell subsets, endowing them with fibrogenic or fibrolytic properties, and the potential of inducing pharmacological reversal even of advanced fibrosis and cirrhosis.

Proof of antifibrotic activity of a given drug is currently based on a significantly ameliorated histological fibrosis score vs the control group in follow up biopsies. Given the high sampling variability of liver biopsy, this requires longterm studies in large cohorts of patients stratified according to intermediate or advanced fibrosis stage and genetic or e.g. metabolic risk factors. The stakeholders, including regulatory authorities, recognize this problem and are in an open dialogue to accept emerging surrogate markers of disease progression towards cirrhosis, decompensation, HCC or death. Such markers need to be biologically plausible and require validation in (ongoing but also retrospective) studies based on liver biopsy. Noninvasive biomarkers for the quantification of liver fibrosis and especially fibrogenesis, i.e., de novo formation of scar tissue, are urgently needed. Current imaging technologies and serum markers permit stratification of patients according to mild vs significant fibrosis (e.g., F0-1 vs. F2-4) or for a reliable diagnosis of cirrhosis, but fail to reliably differentiate one stage-differences. Serum fibrosis markers are either direct tests that are related to the metabolism of the extracellular matrix (ECM), indirect tests that measure parameters of liver function, or a combination of both. Direct markers, such as for the aminoterminal propeptide of procollagen type III (P3NP), collagen type IV, tissue inhibitor of metalloproteinases (TIMP)-1 and hyaluronan (HA), and their combinations correlate with fibrosis stage. Combinations of indirect (or direct and indirect) markers, such as Fibrotest and Fibrometer correlate with fibrosis stage and are in routine clinical use, especially in France. The ELF-panel that consists of P3NP, TIMP-1 and HA may also at least partly reflect fibrogenesis, and is presently favoured by regulatory authorities as a surrogate of fibrosis progression. Still, precision for staging is too low and the ability to reflect fibrogenesis too unproven to qualify these tests as valid surrogates of treatment efficacy in clinical trials of antifibrotic agents.
Recently, novel serum markers have been developed that appear to truly reflect fibrogenesis and even predict therapeutic response to antifibrotic intervention, as evidenced in clinical studies with antifibrotic agents and high quality biopsy follow up. One such marker is Pro-C3, a fragment of procollagen type III that is exclusively released when collagen fibrils are deposited and that does not also mirror collagen type III breakdown, as does the conventional P3NP. Another novel marker is Pro-C6, the carboxyterminal propeptide of the 3-chain of procollagen type VI, an ECM peptide that serves as an adipokine (endotrophin). Pro-C6 reflects not only liver and adipose tissue fibrogenesis, but also insulin resistance, qualifying it as key marker in studies of patients with fibrotic NASH. Other novel markers derive from cell surface molecules involved in the processing of the ECM, reflecting either fibrogenesis or fibrolysis, i.e., the removal of ECM from scar tissue. Apart from serum biomarkers, there is also progress in quantitative imaging of both fibrosis and fibrogenesis, using radioligands that either bind to fibrillary collagen or to surface receptors of cells that drive liver fibrogenesis, such as activated cholangiocytes and myofibroblasts/hepatic stellate cells. These recent developments promise to permit shorter and smaller proof-of-concept trials. Moreover, they will facilitate personalized antifibrotic therapies, employing an individualized dose titration and use of drug combinations for improved efficacy and reduced side effects.

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IDEAL STUDY DESIGNS FOR ANTIFIBROTIC THERAPIES AND CURRENT INDUSTRY ACTIVITIES

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Great excitement about potential antifibrotic therapies is reflected in the growing list of agents being tested in animals and humans, although none has yet been approved for clinical usage and obstacles remain. Moreover, although key pathways of hepatic stellate cell (HSC) activation and fibrosis are attractive targets and are common to all etiologies of chronic injury, disease-specific pathways and patterns of fibrosis progression have also been described. The overwhelming disease focus of current antifibrotic drug trials is NASH, based on the large potential population at risk and the emerging clarity about potential targets. Another key unmet need is sclerosing cholangitis, where the basis for the biliary injury and progressive fibrosis, the ideal therapeutic targets, and the determinants of cholangiocarcinoma risk are all obscure.

Current obstacles to antifibrotic therapy in NASH, include: 1) A lack of consensus on the most important and responsive therapeutic targets in the disease. There is no hierarchy of pathogenic events, so that defining “driver” pathways remains difficult; 2) Animal models are imperfect and not standardized, so that it is unclear how robust preclinical data must be in order to predict responsiveness in humans; 3) Non-invasive surrogates that predict disease progression or response to therapy are rapidly developing but not yet widely validated or sufficient to serve as primary endpoints in phase 3 trials; 4) Testing of antifibrotic drugs in non-cirrhotic patients may need to be unrealistically lengthy if a reduction in clinical events is set as a trial endpoint, whereas drug trials in patients with advanced fibrosis may make it more difficult for a drug to yield a therapeutic response and establish its safety; 5) Antifibrotic therapies for non-viral diseases like NASH must be effective in the face of ongoing injury since, unlike in viral hepatitis, the primary disease may not be curable.

One approach to overcoming the limitation of rodent models is to perform comprehensive transcriptomic analysis to determine whether gene expression in models aligns with human disease gene expression data. The development of non-invasive biomarkers as surrogates for liver biopsy that reflect changes in fibrogenic activity or fibrosis deposition is also a high priority. Subject selection and stratification are also key elements in antifibrotic drug trial design. Risk factors such as genetic factors, age, gender, alcohol use, coffee consumption and metabolic syndrome should be quantified at the time of enrollment. Importantly, different therapies may have differing efficacy depending on the stage of disease. For example, drugs targeting inflammation and cell injury are likely to have the most benefit at earlier and intermediate stages, whereas drugs targeting mechanisms that are more critical
in advanced fibrosis may be better suited for testing in patients with cirrhosis.
The ideal clinical trial for an antifibrotic will need to: 1) Optimize selection of a treatment population by using genetic or circulating markers to stratify based on risk of progression; Establish other markers of progression risk; 2) Attack on molecular targets that are critical to disease pathogenesis, with strong validation in human liver and use of animal models that recapitulate features of human disease; 3) Establish and apply validated biomarkers that provide early and reliable readouts of drug efficacy and correlate with long term outcomes.
The slow, chronic course of liver disease requires drugs to be highly tolerable, and easily administered; thus, oral administration is preferred over parenteral therapies. However, in patients with more advanced disease at risk of clinical decompensation, parenteral administration of drugs might be acceptable.
While there are already dozens of trials being launched, pharma and biotech companies need to mitigate risk by seeking agents that have very strong preclinical proof-of-concept, excellent safety and tolerability, and a clear path to regulatory approval based on endpoints acceptable to regulatory agencies. One approach to streamlining this path is to repurpose drugs developed for other indications that have already have good safety and clinical data and a strong rationale for testing in fibrosis – such agents could typically move directly to Phase 2 trials. Another approach is to seek combinations of drugs that attack separate elements of disease pathogenesis. Finally, the establishment in 2014 of the Liver Forum, an all-stakeholder group of experts from academia, industry, regulatory and patient groups, modeled after similar efforts in HIV and HCV therapies, promises to enhance collaboration, standardize terminology and trial design, and improve transparency in the field, thereby accelerating the testing and approval of antifibrotic therapies.

Disclosure of Interest: None Declared
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PROGRESSIVE FIBROSIS IS DRIVEN BY GENETIC
PREDISPOSITION, ALLO-IMMUNITY, AND
INFLAMMATION IN PEDIATRIC LT RECIPIENTS

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Introduction: Protocol biopsies (PB) from stable liver transplant (LT) recipient children frequently exhibit idiopathic fibrosis (i.e. in absence of LT-related complications). This unexplained progressive fibrosis may become an indication of re-transplantation in the near future.

Aims: To determine predisposing factors of idiopathic allograft fibrosis.

Material and Methods: After screening 235 LT recipients, 89 stable and complication-free children with a total of 281 serial PB’s and paired HLA antibody data were included. PB’s were taken at 1-2, 2-3, 3-5, 5-7 and 7-10 years post-LT and evaluated for inflammation, fibrosis using the LAFSc system. Evolutions of fibrosis, inflammation and their predisposing factors were analyzed using cumulative logistic mixed effect model. A logistic regression model was built to assess predisposing factors for development of class II Donor Specific HLA Antibodies (DSA).

Results: HLA DRB1*03/04 allele and class II DSA are significantly associated with portal fibrosis (OR=2.28, p=0.03, and OR=5.84, p=0.03, respectively). Portal fibrosis severity is variable with time and strongly correlated to preceding portal inflammation (p<0.01). Portal inflammation is predisposed by class II DSA (OR=4.77, p=0.02), non-HLA antibodies (OR=2.31, p=0.01) and is persistent in successive PB (p<0.01). Unlike portal area, central fibrosis is not amenable to change. Non-portal fibrosis is not predisposed by lobular inflammation. Lobular inflammation is associated with non-HLA antibodies and is persistent in successive PB’s.
**Conclusions:** This is the first study that utilizes serial PB’s to delineate the evolution of idiopathic fibrosis, inflammation in the allograft and explores their respective predictors. We conclusively demonstrate that allograft inflammation eventually results in fibrosis. The inflammation is associated with the presence of post-LT class II DSA and non-HLA antibodies. Genetic predisposition for fibrosis is imparted by presence of HLA DRB1*03/04 allele.

**Disclosure of Interest:** None Declared
RELAXIN-COATED IRON-OXIDE MAGNETIC NANOPARTICLES AS A NOVEL THERANOSTIC APPROACH FOR DIAGNOSIS AND TREATMENT OF LIVER FIBROSIS

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Introduction: Hepatic fibrosis is a growing health problem with no effective and clinically approved therapy. Hepatic stellate cells (HSCs) are the key cells involved in the pathogenesis of liver fibrosis. Upon activation, HSCs undergo morphological and functional changes, and are transformed into contractile ECM-producing myofibroblasts leading to scar tissue formation. HSCs contraction contributes significantly to the portal hypertension thereby further impairing the liver function. Relaxin (RLN) has been shown to inhibit HSC activation and contraction thereby ameliorate liver fibrosis and portal hypertension. However, RLN has very poor pharmacokinetics and administration of high or frequent doses can lead to detrimental side effects due to vasodilation.

Aims: In this study, we aimed to develop a nanoparticle-based delivery system to improve pharmacokinetics and therapeutic efficacy of RLN for the diagnosis and treatment of liver fibrosis.

Material and Methods: We conjugated human RLN-2 to PEGylated superparamagnetic iron–oxide nanoparticles (RLN-MNP) and characterized the size, charge and stability. We examined RLN-MNP for RLN conjugation efficiency using Dot-Blot and HSCs-specific binding/uptake. We analysed RLN receptor (RXFP1) expression on activated HSCs and CCl4-induced liver fibrosis mouse model. Finally, we assessed the effects of RLN-MNP on human HSCs in vitro and CCl4-induced advanced 8-weeks liver fibrosis mouse model in vivo.

Results: RLN-MNP was successfully synthesized and remained stable at 4°C. RLN-MNP showed specific binding and uptake to TGFβ-activated human HSCs while MNP alone showed no binding/uptake. In vitro, RLN-MNP and unconjugated RLN significantly
inhibited TGFβ-induced 3D-collagen gel contraction and HSCs migration. Significant up-regulation of RXFP1 in TGFβ-activated HSCs and CCl4-induced liver fibrosis mouse model was observed. *In vivo* in established liver fibrosis mouse model, both RLN and RLN-MNP strongly attenuated fibrosis by inhibiting HSC activation, ECM production and angiogenesis. Importantly, RLN-MNP, but not unconjugated RLN, increased Nitric oxide release by significant up-regulation of iNOS. On the other hand, unconjugated RLN induced systemic side effects by inducing systemic NO release (in serum) while RLN-MNP did not. *In vivo* studies for MRI and portal hypertension are currently ongoing.

**Conclusions:** This study presents a novel strategy to deliver RLN specifically to HSCs, key pathogenic cells involved in liver fibrogenesis, for the diagnosis and treatment of liver fibrosis.

**Disclosure of Interest:** None Declared
IL-13 DRIVES DISTINCT CELLULAR PATHWAYS DIRECTING DUCTULAR REACTION, STEATOSIS, AND FIBROSIS

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Introduction: Fibroproliferative diseases are a major cause of morbidity and mortality that affect nearly every organ system in the body. Recent studies have suggested that type-2 cytokines are critically involved in both fibrogenesis and tissue repair; nevertheless, the mechanisms and cell types that regulate tissue regeneration versus pathological fibrosis are not well understood, particularly in the liver.

Aims: Therefore, we aimed to elucidate the cell-specific role of the type-2 effector cytokine IL-13 in driving hepatic fibrosis and associated pathologies, with particular focus on defining the mechanisms underlying the correlation between fibrosis and dysregulated regeneration of bile ducts known as ductular reaction (DR).

Material and Methods: To achieve this, we developed several conditional knockout mice with IL-13 signalling disrupted in hepatocytes, ductal cells, or fibroblasts, and infected these mice with S. mansoni, a parasite affecting over 300 million individuals that induces progressive IL-13 driven fibrosis. Furthermore, to determine if IL-13 alone is sufficient to drive the pathologies observed during S. mansoni infection, we utilized hydrodynamic delivery of IL-13 overexpression plasmids to induce liver-specific IL-13 secretion in the absence of infection.

Results: Ablation of IL-13 signalling in ductal cells using Krt19-CreERT2 mice completely eliminated DR and steatosis, yet had no effect on fibrosis, revealing that IL-13 signalling in ductal cells directly drives DR and steatosis without affecting the progression of fibrosis. Ablation of IL-13 signalling in fibroblasts using PDGFRB-Cre mice resulted in reduction of fibrosis to that of controls (Figure 1), without affecting the development of DR and
steatosis. Furthermore, plasmid overexpression of IL-13 recapitulated pathology observed in infected mice, demonstrating the central role of this cytokine in the progression of hepatic fibrosis.

**Conclusions:** These findings confirm that IL-13 initiates fibrosis by directly activating PDGFRB+ fibroblasts, the first study demonstrating such *in vivo*. Furthermore, we have demonstrated that fibrosis and associated DR and steatosis arise independently due to direct IL-13 signalling on fibroblasts and ductal cells, illustrating that the function of this cytokine is much broader than previously appreciated. We anticipate that these findings will prompt a new wave of clinical research investigating the therapeutic potential of interleukin-13 modulation in the treatment of various aspects of liver disease.

**Figure:**

![Image of fibrosis severity and hydroxyproline content](image)

**Disclosure of Interest:** None Declared
YI-MP-151

THE I148M PNPLA3 VARIANT AS NOVEL KEY PLAYER MODULATING THE PRO-FIBROGENIC PHENOTYPE OF HUMAN HEPATIC STELLATE CELLS

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Introduction: Adiponutrin (PNPLA3) is a nutritionally regulated protein that displays triglyceride hydrolase and/or lysophosphatidic acid acyltransferase activities. A PNPLA3 genetic variant, known as the I148M isoform, is strongly associated with hepatic steatosis and its progression toward nonalcoholic steatohepatitis (NASH), cirrhosis and cancer. Hepatic stellate cells (HSC) are key players in the development of liver fibrosis, but little is known about the role of PNPLA3 and its variant I148M in HSC metabolism and fibrogenesis.

Aims: To explore how PNPLA3 expression is regulated during HSC activation and investigate its impact on hepatic fibrogenesis.

Material and Methods: Primary human HSC have been isolated from liver unsuitable for transplantation. We stably transfected LX2 cells with plasmids carrying either the wild type or the I148M PNPLA3 isoform. We performed western blot analysis, real-time PCR, MTT, Gel shift and migration assays.

Results: Transcriptional and translational levels of PNPLA3 increases during primary human HSC activation. We analyzed our primary human HSC in two groups, according to PNPLA3 genotype (WT=wild type, I148M=allele variant). Untreated HSC carrying the I148M PNPLA3 variant displayed significantly higher expression of pro-inflammatory cytokines, such as Rantes and GM-CSF, which enhanced macrophages migration. To investigate the molecular mechanisms explaining the I148M phenotype, we generated two stable cell lines overexpressing the WT and I148M PNPLA3 isoforms. In addition to increased production and secretion of pro-inflammatory cytokines the I148M cells proliferated and were more fibrogenic than their WT controls. c-Jun kinase (JNK) as well as AP-1 were activated in HSC carrying the variant PNPLA3, which explained the
cytokines production. Interestingly, we established that the AP-1 promoter activity was increased in I148M expressing cells. PNPLA3 is a key player in lipid metabolism and PPARγ is known to regulate AP-1 activity. We therefore explored PPARγ expression and functionality in cell line stably expressing PNPLA3 WT and I148M isoforms. Despite a normal PPARγ ligands abundance, the transcriptional activity of the nuclear receptor was lowered in I148M expressing cells due to Serine 84 phosphorylation mediated by JNK.

**Conclusions:** All together our data described how PNPLA3 is regulated during HSC activation by inhibition of PPARγ activity which leads to higher AP-1 and cytokines release in I148M variant thus promoting fibrosis progression.

**Disclosure of Interest:** None Declared
**Introduction:** Liver fibrosis is the final end stage of most chronic liver diseases caused by various etiologies. This process leads to the progressive accumulation of extracellular matrix (ECM) components in an attempt to limit hepatic damage. Hepatic stellate cells (HSCs) are the major source of ECM and key mediators of fibrogenesis. MerTK is a receptor tyrosine kinase that have immunologic and oncogenic properties. In a recent GWAS study a SNP of Mertk (rs4374383 G>A) has been associated with fibrosis severity in patients with chronic hepatitis C, but currently there is no evidences on the role of MerTK in the hepatic fibrosis process.

**Aims:** To assess the potential role of Mertk in the fibrogenic process.

**Material and Methods:** Human HSCs were isolated from livers and cultured on plastic. The inhibitor used was UNC569. C57BL6/J mice were treated with CCl4 for 6 weeks. Balb/C mice were fed with a methionine and choline deficient (MCD) diet for 8 weeks. Intrahepatic gene expression was assayed by qPCR.

**Results:** Mertk rs4374383 AA genotype was associated with a lower prevalence of clinically significant fibrosis in patients with NAFLD and a decreased expression of MerTK. In two mouse models of liver fibrosis (CCl4 and MCD diet) we observed an increased hepatic expression of MerTK gene. In HSCs MerTK was found highly expressed and stimulation with a Gas6, a MerTK ligand, resulted in a time-dependent activation of ERK1/2. Moreover, Gas6 enhances cell motility and this stimulatory effect was abrogated by pretreatment with MerTK inhibitor UNC569. Exposure of HSC to UNC569 or gene silencing resulted in a decrease of cell viability. Analysis of apoptotic
markers indicated that the reduction of cell viability was due to programmed cell death. Gas6 treatment significantly increased the expression of procollagen I and this effect was blocked by co-exposure of HSCs with UNC569. A similar effect was observed in MerTK-silenced cells, suggesting a direct profibrogenic role of MerTK. Analysis of mRNA levels in liver specimens from patients with NAFLD, presenting different degree of liver fibrosis, showed that MerTK expression correlates with the score of fibrosis observed. Finally, a higher expression level of MerTK mRNA was observed in cirrhotic liver tissue compare to HCC, suggesting the pivotal role of MerTK also in advanced stage of fibrosis.

**Conclusions:** These data indicate a profibrogenic role of Gas6/MerTK pathway and additional studies are needed to characterize this pathway.

**Disclosure of Interest:** None Declared
YI-MP-I22

CIRCULATING ENDOTHELIAL PROGENITOR CELLS OF CIRRHOTICS ENHANCE ANGIOGENESIS AND LIVER FIBROSIS IN BILE DUCT-LIGATED RATS

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Introduction: Previous in vitro studies from our lab have demonstrated that in comparison to healthy control-derived endothelial progenitor cells (EPCs), cirrhotic patient-derived EPCs secrete higher proangiogenic factors such as VEGF and PDGF and lead to greater enhancement of angiogenesis via interaction with resident liver endothelial cells.

Aims: To evaluate the effect of cirrhotic and control EPCs on hepatic angiogenesis and fibrosis in vivo.

Material and Methods: Animal models were prepared by identifying and ligating the bile duct. EPCs isolated from cirrhotic and healthy human blood were cultured ex vivo and transplanted in different groups of BDL rats via tail vein thrice a week for two weeks. Liver functions and fibrosis was evaluated by histopathology in healthy-EPC and cirrhotic EPC-treated rats. Expression of important angiogenic markers, VEGF and CD31, fibrogenic markers, alpha-SMA and TGFbeta and regeneration markers, PCNA and HGF was studied in different groups by western blot, immunohistochemistry and real time PCR. In HSCs were co-cultured with conditioned media from control and cirrhotic EPCs. Proliferation of HSCs analyzed by brdu assays and the levels of important angiogenic factor secreted by HSCs, vascular endothelial growth factor (VEGF) was evaluated by ELISA in presence of EPC-CM from patients and controls.

Results: We found increased fibrosis in cirrhotic-EPC-treated rats vs healthy EPC-treated. IHC, RTPCR and western blot data showed an enhancement of both fibrosis and angiogenesis markers, alpha-SMA and CD31 in cirrhotic EPC-treated rats vs healthy EPC-treated rats(P<0.01). In regeneration markers, maximum PCNA positivity was obtained in healthy EPC-treated rats while HGF was more in both healthy and cirrhotic
EPC-treated rats. No significant differences in the liver functions among different groups of BDL rats observed. HSCs were co-cultured with EPC-CM from cirrhotic patients showed significantly increased proliferation in comparison to that observed in HSCs alone (P< 0.05). However, VEGF levels in HSCs didn’t show a significant change in presence of EPC-CM from the patients and controls.

Conclusions: This suggests that EPCs contribute to in vivo angiogenesis in injured liver and that cirrhotic EPCs have enhanced angiogenic and fibrotic functions as compared to the healthy EPCs.

Disclosure of Interest: None Declared
THERAPEUTIC INTERVENTION WITH EVASIN-4 ATTENUATES LIVER FIBROGENESIS AND THE PROGRESSION OF HEPATOCELLULAR CARCINOMA

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Introduction: Hepatocellular carcinoma (HCC) which is the consequence of malignant transformation of hepatocytes, is often based on chronic inflammation, resulting in liver fibrogenesis. During this process chemokines play a crucial role by attracting immune cells.

Aims: In the current study, we investigated whether therapeutic intervention with Evasin-4 – a CCL5/RANTES inhibitor – affects the development of hepatic fibrogenesis. Additionally, the role of CCL5 in the development of fibrosis and tumour initiation and progression was studied in two independent murine models of inflammation triggering HCC development. As translational aspect, we analyzed a well-defined patient’s cohort suffering from chronic liver disease (CLD).

Material and Methods: For therapeutic intervention Evasin-4 was injected either for 24 hours or for 8 weeks. The role of CCL5 in inflammation, fibrosis, tumour initiation and progression was analysed in different cell populations using NEMO<sup>Δhepa</sup>/CCL5<sup>−/−</sup> mice and subsequent bone marrow transplantation (BMT) studies.

Results: In CLD patients, CCL5 expression correlated with inflammation stage and fibrosis grade. Interestingly, despite CCR1 and CCR3, CCR5 was upregulated in this cohort. Genetic inactivation of CCL5 in NEMO<sup>Δhepa</sup> mice diminished hepatocyte apoptosis, compensatory proliferation and fibrogenesis due to reduced immune cell infiltration.
In this context, especially CD45+ Ly6G+ granulocytes, pro-inflammatory monocytes, and T-cells were decreased in NEMO\textsuperscript{Δhepa}/CCL5\textsuperscript{-/-} livers. As a consequence, late stage NEMO\textsuperscript{Δhepa}/CCL5\textsuperscript{-/-} mice displayed smaller and less malignant tumours, characterized by significantly reduced proliferative capacity and less pronounced angiogenesis in comparison with NEMO\textsuperscript{Δhepa} tumors. Mechanistically, we identified hematopoietic cells as main source of CCL5. Short term Evasin-4 treatment significantly reduced the infiltration of CD45+ Ly6G+ granulocytes. Subsequently, 8 week treatment of NEMO\textsuperscript{Δhepa} mice resulted in a significant improvement of liver fibrosis confirmed by reduction of established fibrosis markers.

**Conclusions:** Therapeutic modulation of the CCL5/RANTES pathway with Evasin-4 significantly attenuated the development of hepatic fibrosis. Deletion of CCL5 in NEMO\textsuperscript{Δhepa} mice leads to the amelioration of hepatic fibrogenesis and HCC progression. These results indicate that CCL5 might be an attractive target for the treatment of chronic liver disease.

**Disclosure of Interest:** None Declared
ePOSTER ABSTRACTS
INTERLEUKIN-33/INTERLEUKIN-33R SIGNALING PROMOTES LIVER INFLAMMATION AND FIBROGENESIS IN OBESOGENIC MODEL OF NONALCOHOLIC STEATOHEPATITIS IN MICE

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Introduction: Cytokines have been shown to modulate liver fibrosis, but the role of IL-33/IL-33R(ST2) signaling pathway in obesity-associated liver pathology is only partially understood.

Aims: In order to dissect the role of IL33/ST2 axis we used obesogenic model of nonalcoholic steatohepatitis (NASH) in C57BL/6 and BALB/c mice, the prototypical Th1 and Th2 strains.

Material and Methods: Male, 8-week old ST2 deficient (ST2−/−), wild-type (WT) BALB/c mice and C57Bl/6 mice were placed on high-fat diet (HFD; 60% kcal fat) or standard diet (10% kcal fat) for 24 weeks. After 10 weeks on HFD, one group of C57Bl/6 mice were injected intraperitoneally with murine recombinant IL-33 (0.5 μg) five times every other day. Histological, immunophenotypic and gene expression analyses were performed.

Results: HFD enhanced liver inflammation and fibrosis in C57Bl/6 mice, associated with more numerous pro-inflammatory CD11b+Ly6Chi monocytes, triple positive F4/80+CD11b+CD11c+ and IL-1β-expressing F4/80+ macrophages (Mφ) and increased expression of procollagen α1, IL-33, IL-13 and TGF-β mRNA compared to mice on standard diet. IL-33 administration in vivo enhanced liver inflammation and fibrosis and...
increased the number of hepatic CD11b+ myeloid cells expressing ST2 and IL-13. In BALBc, deletion of ST2 markedly reduced HFD-induced liver steatosis and inflammation, accompanied with lower expression of CD36, LXRα, PPAR-γ and decreased number of CD68+ Mφ and CD11c+ dendritic cells (DCs). Further, HFD-fed ST2-/- mice had lower collagen deposition in livers associated with less numerous profibrotic CD11b+Ly6Clow monocytes and CD4+IL-17+ T cells and lower hepatic procollagen α1, IL-33 and IL-13 mRNA expression, and lower serum levels of IL-33 and IL-13, while the serum levels and hepatic TGF-β mRNA expression were similar between ST2-/- and WT mice. In an additional experiment in which we used HFD/fructosis diet, fibrosis exacerbated in WT mice, but diet had no effect on ST2-/- mice. In contrast, HFD-fed ST2-/- mice exhibited higher weight gain, amount of visceral adipose tissue (VAT), and enhanced VAT inflammation with higher percentages of VAT associated CD11c+ DCs, IFNγ and IL-17 expressing T cells, and CXCR3+ T cells compared to diet-matched WT mice.

Conclusions: IL-33/ST2/IL-13 pathway has an important role in NASH associated fibrosis. Deletion of IL-33R has differential effects on obesity-associated VAT inflammation and liver pathology. IL-33/ST2 signaling is involved in obesity-associated liver pathology in Th1 and Th2 dominant strains.

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RELATIONSHIP BETWEEN CA19.9 AND FIBROSIS IN A COHORT OF PATIENTS WITH VIRAL HEPATITIS AND WITHOUT MALIGNANCIES. CA19.9 LEVELS REFLECT THE GRAVITY OF FIBROSIS

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Introduction: Several previous studies have shown that CA19-9 serum level can increase in patients affected by non-malignant diseases. In particular, in patients with idiopathic interstitial pneumonia, idiopathic pulmonary fibrosis and collagen-associated diseases, but also in liver diseases as for example fibrosis and cirrhosis.

Aims: We want report our experience in patients with chronic viral hepatitis but without malignant disease, cholestasis or other chronic diseases that may explain the increase in CA19-9 serum.

Material and Methods: 190 Caucasian patients with viral hepatitis were enrolled (106 males, 84 females) excluding patients with cancer, severe jaundice, chronic airways and kidney diseases, prostatic diseases, autoimmune diseases, and diabetes mellitus. None of the patients consumed alcohol or were taking hepatotoxic drugs. Clinical, hematocemial, virological, diagnostic imaging and histological analysis were performed. Selected patients were randomized into two groups based upon viral etiology. Further CA19-9 evaluations were performed each 6 months for three consecutive years, repeating diagnostic for malignancies.

Results: Data are expressed as mean ± SO. The baseline CA19-9 serum levels (normal value range: 0.0-39.0IU/ml) were 79.7 ±40.2 in group 1 and 83.6 ±42.9 in group 2. Further CA19-9 evaluations were performed each 6 months for three consecutive years, and median follow-up CA19-9 level was 76.8 ± 44.1 in group 1 and 79.3 ± 49.5 in group 2. Mean ± SO serum alpha-fetoprotein was 14.3±11.2 in group 1 and 14.8±10.4 in group 2 (normal value: up to 5.8 IU/ml). All patients were stratified according to Metavir score. The proportion of cirrhotic vs noncirrhotic patients was 48% vs 52% in group 1, and 76%
vs 27% in group 2. In our data 52% of chronic hepatitis C and 49% of chronic hepatitis B showed high levels of CA19-9

**Conclusions:** Increased CA19-9 serum levels are frequent in chronic viral hepatitis. This does not indicate concurrent neoplasia, but correlates with the grade of liver fibrosis. Increase in CA19-9 is higher in patients with HCV infection compared with HBV, thus probably related to the fibrogenic HCV properties. Further investigations may clarify if CA19-9 assumes the role of an indirect marker of hepatic fibrosis. We propose that CA19-9 can be used in combination with the other markers already in use, in order to increase the diagnostic accuracy of the available tests.

**Figure:**

<table>
<thead>
<tr>
<th>Grade of Fibrosis</th>
<th>Level of CA19.9 Group 1</th>
<th>Level of CA19.9 Group 2</th>
</tr>
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<tr>
<td>F0</td>
<td>/</td>
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<td>F1-F3</td>
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<td>75.1±11.2</td>
</tr>
<tr>
<td>F4</td>
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**Disclosure of Interest:** None Declared
Screen 3: YI-MP-205

GALECTIN-3 PLAYS AN IMPORTANT ROLE IN EARLY STAGES OF NON-ALCOHOLIC STEATOHEPATITIS BY PROMOTING ACTIVATION OF NATURAL KILLER CELLS

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Introduction: Gal-3 participates in the pathogenesis of liver inflammation and fibrosis, but its importance in NK cell function in early stages of obesity-associated non-alcoholic steatohepatitis (NASH) is incompletely defined.

Aims: In this study, we aimed to dissect the role of Gal-3 in early inflammatory response and natural killer (NK) cells activation in obesogenic mouse model of NASH.

Material and Methods: Gal-3-deficient (LGALS3-/-) and wild-type (WT) male C57Bl/6 mice received HFD (60% kcal fat) or standard chow diet for 4 weeks and immunophenotypic analyses of mononuclear cells in the liver were performed.

Results: Compared to LGALS3-/- mice, HFD-fed WT mice had significantly higher inflammatory score in the liver after 4 weeks on HFD. Pronounced inflammatory response in liver was associated with increased proportions of hepatic effector NK cells and proinflammatory macrophages. The percentage of NK1.1+IFN-γ+ and NK1.1+CD27+CD11b+ effector cells was significantly higher in WT mice compared with LGALS3-/- mice, both on HFD. Furthermore, HFD significantly increased the proportion of activated CD69+ NK cells in WT mice compared with diet matched LGALS3-/- mice. Additionally, HFD significantly increased immature CD27-CD11b+ NK cells in both genotypes, with no difference between WT and LGALS3-/- mice. Frequency of CD11b+CD27- NK cells that have reduced effector function and NK cells expressing inhibitory KLRG1+ molecules did not differ between two genotypes of mice. The presence of NK1.1+ cells expressing NCR-1 molecules, known to down-regulate liver fibrosis through the killing of activated hepatic stellate cells (HSCs), were significantly increased in LGALS3-/- mice compared with WT mice on both diets. In addition,
proinflammatory CD11b+Ly6Chi monocytes and M1-macrophages were present in greater proportions in livers of HFD-fed WT compared to diet-matched LGALS3−/− mice.

**Conclusions:** The obtained data suggest an important regulatory role for Gal-3 in the development of early stage of liver inflammation mediated by NK cells and macrophages in NASH. Gal-3 inhibition can be novel therapeutic approach in obesity-associated liver inflammation and fibrosis.

**Disclosure of Interest:** None Declared
ALBI SCORE AS NON-INVASIVE BIOMARKER OF CIRRHOSIS IN CHRONIC HEPATITIS C

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Introduction: Chronic hepatitis C (CHC) is a major cause of cirrhosis and hepatocellular carcinoma (HCC). Further, information on the stage of liver fibrosis is fundamental in managing CHC patients. Several scores, based on routine laboratory tests, have been proposed to assess disease progression. Interestingly, researchers have recently found a simpler model based on albumin and bilirubin levels denominated ALBI score, which can help to predict mortality in patients with HCC or primary biliary cirrhosis. However, little is known about the utility of ALBI score to discriminate cirrhosis in CHC patients.

Aims: This study was conducted with the aim of evaluating the serum levels of albumin and bilirubin in cirrhotic and non-cirrhotic CHC patients in order to investigate the prognostic significance of the ALBI score as potential non-invasive biomarker of CHC progression.

Material and Methods: A total of 169 CHC patients were included in our study. Albumin and bilirubin data were extracted from routine tests of blood samples that were collected from all patients on the same day that they had undergone liver biopsy. Fibrosis staging was determined according to METAVIR system. ALBI score was calculated according to the described formula and its correlation with fibrosis was investigated by Spearman test. Cirrhotic and non-cirrhotic patients were compared using Mann-Whitney non-parametric test. The diagnostic accuracy of ALBI for cirrhosis was examined based on the sensitivity, specificity and area under the receiver operating characteristic curve (AUCROC).

Results: Bilirubin serum levels and ALBI values were significantly increased in cirrhotic patients compared to non-cirrhotic (p<0.001, both). In contrast, expression of albumin was significantly lower in cirrhotic patients (p<0.05). Also, ALBI score was significantly correlated with fibrosis (p=0.002). The optimal cut-off value of ALBI for distinguishing
CHC patients with cirrhosis yielded a sensitivity of 69% and a specificity of 66.4%, with an AUC of 0.705 (p=0.0003).

**Conclusions:** The study shows the known albumin and bilirubin relationship with the progression of CHC to the end stages of liver disease. ALBI score exhibited a moderate diagnostic performance for the detection of cirrhosis but may serve as a simpler and useful non-invasive marker of CHC progression. Additional studies evaluating larger cohorts of patients are warranted to assess the realistic potential of ALBI for discerning CHC-related cirrhosis.

**Disclosure of Interest:** None Declared
EFFICACY OF T1 MAPPING ON GD-EOB-DTPA-ENHANCED MRI FOR STAGING LIVER FIBROSIS IN CHRONIC HEPATITIS B PATIENTS WITH NORMAL ALANINE TRANSAMINASE < 40 IU/L

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Introduction: Chronic hepatitis B (CHB) poses a high risk to liver fibrosis, which may develop into cirrhosis and hepatocellular cancer. Elevated alanine transaminase (ALT) level is a marker of CHB severity and response to treatment. However, several studies have revealed about one fifth of CHB patients with normal ALT concentrations had stages 2-4 fibrosis. Thus, ALT levels in this setting may lead to low positive predictive value of some serum fibrosis markers, such as FIB-4 index (based on age, aspartate aminotransferase, ALT, and platelets). Gd-EOB-DTPA is a liver-specific MRI contrast medium and was reported to be able to predict liver fibrosis stage with high reliability.

Aims: The purpose of this study was to retrospectively assess the diagnostic efficacy of T1 mapping on Gd-EOB-DTPA-enhanced MRI for liver fibrosis staging in CHB patients with normal ALT level.

Material and Methods: This retrospective study included 92 CHB patients (mean age: 54 years; 76 men and 16 women) who underwent Gd-EOB-DTPA-enhanced MRI including T1 mapping. Liver function tests were performed and ALT levels were < 40 IU/L. T1 mapping was performed before and 20-min hepatobiliary phase (HBP) after injection of Gd-EOB-DTPA (Primovist, Bayer-Schering). Liver fibrosis stages were histologically determined according to Scheuer scoring system: S0 (n=18), S1 (n=11), S2 (n=11), S3 (n=11) and S4 (n=41). T1 relaxation times were measured and the reduction rate (Δ%) of the T1 relaxation time was calculated. Spearman’s rank correlation coefficients were calculated and receivers operating characteristic (ROC) curves were built to assess the performance of T1 mapping in identifying liver fibrosis.

Results: The HBP, Δ% of T1 relaxation times showed significant correlations with liver fibrosis stage (rho: 0.71, -0.61, P<0.05). Areas under ROC curves (AUROCs) of pre-
contrast, HBP, Δ% T1 relaxation time for the diagnosis of presence of liver fibrosis (S≥1), significant (S≥2), advanced fibrosis (S≥3) and cirrhosis (S=4) are shown in the Figs 1. The AUROC of HBP and Δ% T1 relaxation time for identification of various degree of fibrosis was significantly greater than pre-contrast T1 relaxation times (P<0.05). The sensitivities, specificities for predicting advanced fibrosis were 82.69% and 85.00% at the cut-off of 212.4 for HBP T1 relaxation times, and were 84.62% and 67.50% at the cut-off of 69.44% for Δ% T1 relaxation times.

**Conclusions:** The Gd-EOB-DTPA-enhanced T1 mapping is accurate for staging liver fibrosis in CHB patients with normal ALT level.

**Figure:**

![Graphs showing sensitivity and specificity for different stages of fibrosis with HBP and Δ% T1 relaxation time compared to pre-T1 mapping.](image)

**Disclosure of Interest:** None Declared
RNAI-MEDIATED INHIBITION OF CYCLIN E1 PROTECTS FROM LIVER FIBROSIS IN MICE

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Introduction: Cyclin E1 (CcnE1) is the regulatory subunit of the Cyclin-dependent Kinase 2 (Cdk2) and a key factor for initiation of cell cycle re-activation. Initiation and progression of liver fibrosis requires proliferation and activation of resting hepatic stellate cells (HSC). We have recently shown that genetic inactivation of CcnE1 prevents activation, proliferation and survival of HSC and protects from liver fibrogenesis.

Aims: The aim of the present study was to translate these findings into pre-clinical application by inhibiting CcnE1 using RNA interference (RNAi).

Material and Methods: The efficiency of several siRNA molecules for inhibiting CcnE1 were tested in murine (GRX) and human (LX2) HSC cell lines and in primary murine and human HSC in vitro. For in vivo applications, we used stabilized CcnE1 siRNA in combination with a liposome-based carrier, which was applied via mild tail vein injection in C57BL/6 wildtype mice. Functionality of CcnE1 siRNA in vivo was tested after Carbontetrachloride (CCl₄)-mediated acute liver injury and in fibrotic livers following 4 week CCl₄ treatment.

Results: HSC cell lines were transfected with CcnE1 siRNA resulting in efficient inhibition of CcnE1. Reduced CcnE1 expression resulted in diminished proliferation and increased cell death. Importantly, comparable results were obtained using murine and human primary HSCs. Delivery of siRNA in mice was optimised and quantified using fluorescence-labelled scrambled siRNA. Transduction rates were 95% in HSC, 75% in hepatocytes, and 50% in CD45 positive cells after single injection. Acute CCl₄ injury substantially induced endogenous CcnE1 expression. Pre-treatment with CcnE1 siRNA reverted CcnE1 expression to baseline levels of healthy mice. Moreover, CcnE1 inhibition was associated with significantly reduced serum transaminases and impaired proliferation.
of hepatocytes and non-parenchymal liver cells. In the chronic CCI₄ model, weekly injection of CcnE1 siRNA significantly limited fibrosis as shown by inhibition of septum formation, decreased Hydroxiproline content and overall reduced liver injury.

**Conclusions:** Our present results demonstrate for the first time that a pre-clinical targeting of CcnE1 using RNAi is feasible and has high anti-fibrotic therapeutic potential in mice and man.

**Disclosure of Interest:** None Declared
SURPRISING PRO-FIBROTIC EFFECT OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN A METHIONINE-CHOLINE DEFICIENT DIET MODEL IS ASSOCIATED WITH A SHIFT IN NATURAL KILLER T CELL POPULATIONS

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Introduction: Macrophage migration inhibitory factor (MIF) is a pleiotropic and inflammatory cytokine with chemokine-like functions. In previous models of hepatotoxic liver injury, MIF shows anti-fibrotic properties via CD74/AMP kinase. Similarly, in non-alcoholic steatohepatitis (NASH) models, MIF exhibits CD74/AMPK-dependent anti-steatotic properties involving alternative macrophage polarization.

Aims: Because steatohepatitis is associated with liver fibrosis, we here studied the role of MIF on liver fibrosis in the methionine-choline deficient (MCD)-diet-model which establishes fibrosis and steatohepatitis within the mice.

Material and Methods: Mif gene-deficient (Mif−/−) and wild-type mice were fed the MCD diet for eight weeks. Fibrosis was analyzed histologically by Sirius Red stainings, by hydroxyproline content and intrahepatic mRNA expression of fibrosis-related genes (aSma, Col1a1, Timp1, Tgfβ1, Mmp2/9). Immune cell recruitment was assessed by flow cytometry and immunohistochemistry. Cytokine profiles and natural killer T (NKT)-cell markers were determined by qRT-PCR.

Results: In contrast to hepatotoxic models, Mif−/− mice showed decreased liver fibrosis after MCD-diet as assessed by Sirius Red stainings and hydroxyproline content. Reduced fibrosis was associated with strong alterations of fibrosis-relevant genes in Mif−/− mice, which is attended by a reduction of stellate cell activation. Flow cytometry revealed no difference in monocyte or lymphocyte infiltration in the livers of Mif−/− and wild-type mice.
However, NKT cells were strongly enhanced in Mif −/− mice. This difference in the NKT cells could not be observed in hepatotoxic liver injury models. Profiling of inflammatory and anti-inflammatory cytokines and NKT-subset markers for type I (iNKT) cells or type II cells demonstrated that hepatic NKT cells in Mif −/− mice were skewed toward type II NKT cells, which inhibit type I NKT-driven liver injury.

**Conclusions:** In our NASH-driven MCD liver fibrosis-model, MIF shows pro-fibrotic properties. These results describe a yet undiscovered relation of MIF and NKT cell subsets. NKT cell subset are skewed towards type II NKT cells by MIF, offering a novel mechanism through which MIF affects liver disease dependent on the pathogenic context.

**Disclosure of Interest:** None Declared
Screen 2: YI-MP-I38

THE GLP-1 RECEPTOR AGONIST LIRAGLUTIDE PROMOTES DE-ACTIVATION OF HEPATIC STELLATE CELLS LEADING TO A MARKED IMPROVEMENT IN THE LIVER MICROVASCULAR DYSFUNCTION OF RATS WITH CHRONIC LIVER DISEASE

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Introduction: Hepatic stellate cells (HSC) play a key role in the development of hepatic microcirculatory dysfunction and fibrogenesis in patients with chronic liver disease (CLD). Glucagon-like peptide 1 (GLP-1) receptor agonists, like liraglutide, are well established in the control of type 2 diabetes and showed anti-inflammatory and anti-oxidant properties.

Aims: To evaluate the effects of Liraglutide on the HSC phenotype and hepatic microvascular function using diverse in vitro, ex vivo and in vivo pre-clinical models of CLD.

Material and Methods: In vitro, Liraglutide (10-50μM; 24h-72h), or vehicle, was administered to primary and immortalized human and rat activated HSC, and to HSC undergoing in vitro activation. Possible synergistic effects with simvastatin (5μM) were tested. HSC phenotype was evaluated using well-established markers (n=3-5 per experimental condition).

In vivo, Liraglutide (0.5mg/kg/day, s.c.), or vehicle, was administered during 15 days to rats with CLD (due to chronic thioacetamide; n=11 per group). In vivo systemic and hepatic hemodynamics (mean arterial pressure, portal pressure, portal blood flow), liver microvascular function (dilatation in response to acetylcholine), fibrosis (Sirius red), HSC phenotype (mRNA and protein markers) and sinusoidal phenotype (fenestrae by SEM) were determined.

Ex vivo, effects of 24h 50μM liraglutide on HSC phenotype were analysed in human precision-cut liver slices (n=3).
**Results:** Liraglutide markedly improved HSC phenotype as demonstrated by down-regulations in α-sma (-80% mRNA, -40% protein) and collagen (-60% mRNA). Its effects were greater in combination with simvastatin (α-sma -65% protein). Liraglutide did not affect HSC viability but significantly diminished cell proliferation (-37% proliferation, -65% PDGFRβ). All p<0.05.

CLD-rats receiving liraglutide exhibited significantly lower portal pressure than those treated with vehicle (11.6±0.8 vs. 9.3±1.0 mmHg; -20%) due to a reduction in the intrahepatic vascular resistance (-24%), that was accompanied by marked improvements in the hepatic vascular function (+23% vasorelaxation), fibrosis (-22%), HSC phenotype (α-sma -20%, collagen -48%, PDGFRβ -27%) and sinusoidal phenotype (fenestrae: +71% porosity & +61% frequency).

Anti-fibrotic effects of liraglutide were confirmed in human liver tissue (-41% in α-SMA).

**Conclusions:** The obtained results indicate the potential beneficial effects of liraglutide used as an agent to promote the regression of chronic liver disease.

**Disclosure of Interest:** None Declared
Screen 3: YI-MP-145

THE PLATELET-DERIVED CHEMOKINE CXCL4 EXERTS PROTECTIVE ROLE IN NON-ALCOHOLIC STEATOHEPATITIS (NASH) IN VIVO

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Introduction: Non-alcoholic steatohepatitis (NASH) is the third most common reason for liver transplantations in developing countries and one of the fastest growing medical problems. It is known that platelets are involved in non-alcoholic fatty liver disease. The significance of CXCL4, one of the most abundant chemokines in platelets, in NASH development and fibrosis progression remains unclear.

Aims: We investigated the role of the chemokine (C-X-C motif) ligand 4 (CXCL4), also known as platelet factor 4 (PF4), in two different diet-induced NASH mouse models the high fat (HF)-diet and the methionine and choline deficient (MCD) diet model.

Material and Methods: Constitutive wildtype (WT) and CXCL4 knockout (KO) mice were fed HF-diet for 14 weeks or MCD-diet for 6 weeks.

Results: After 14 weeks of HF treatment CXCL4 deficient animals showed typical features of the metabolic syndrome compared to WT controls. Especially a significant increase in bodyweight and elevated leptin mRNA expression levels were found. Consistent with this KO mice displayed massive fatty liver degeneration with more pronounced histomorphological changes and a significantly increased hepatic triglyceride and cholesterol content in the HF and in the MCD model. In both diet-induced steatosis models NASH progression was further reflected by higher serum transaminase levels and stronger hepatic immune cell infiltration. Especially t cells, neutrophils and inflammatory macrophages were increased in livers of mice lacking CXCL4 compared to controls. Similar results were found when analysing the immune cell infiltration in epididymal white adipose tissue (eWAT). Namely higher numbers of different t cell populations and neutrophils in CXCL4 KO mice. Those inflammatory changes resulted in a worsened glucose tolerance as it occurs in human obesity and type 2 diabetes. Finally CXCL4 KO mice displayed earlier and faster fibrosis progression by stronger collagen accumulation.
compared to WT mice. CXCL4 deficient animals showed a significant increase in Sirius Red stained collagen fibres (Fig. 1) and elevated mRNA levels of transforming growth factor β (TGFβ) and Collagen 1A1.

Conclusions: CXCL4 has a protective function in HF- and MCD-induced fatty liver disease since a deficiency for this chemokine resulted in faster and stronger development of dietinduced NASH. Therefore PF4 provides a potential therapeutic agent for the intervention during steatohepatitis development and progression.

Figure:

![Figure 1: Increased hepatic fibrosis in MCD- and HF-diet treated CXCL4−/− mice](image)

Representative Sirius red stained liver paraffin sections of WT and CXCL4−/− mice 6 weeks of MCD-diet and 14 weeks of HF-diet. (Scale bars 100μm, magnification 200x).

Disclosure of Interest: None Declared
THE UNFOLDED PROTEIN RESPONSE IS A VERY EARLY EVENT DURING HEPATIC STELLATE CELL ACTIVATION


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Introduction: Liver fibrosis or scarring of the liver is the consequence of prolonged hepatocytic damage that results in persisting hepatic stellate cell (HSC) activation. This makes stellate cells the primary targets for anti-fibrotic therapy and emphasizes the need to understand how HSCs contribute to fibrosis development. The unfolded protein response (UPR) is a cellular response related to ER stress. Chemical induction of ER stress has been shown to affect HSC activation.

Aims: In this study we investigated whether endogenous ER stress is essential for the earliest phases of mouse HSC (mHSC) activation.

Material and Methods: In vitro and in vivo activated HSCs were analyzed for ER stress by qPCR and immunohistochemistry and in WT and JNK-Knock-out mice.

Results: The ER stress markers, XBP1spliced, BiP and Chop, showed an early peak in mRNA expression already 10h after seeding primary mouse HSCs on plastic culture dishes, followed by a decreased expression at 24h. Up to 10 days, the expression levels did not differ from the 24h time point. This increased ER stress could also be seen in freshly isolated HSCs from mice 10h after 1 CCl4 injection suggesting that ER stress is an early event of HSC activation also in vivo. HSCs cultured as 3D spheroids showed prevention of early ER stress and expression of activation markers was inhibited compared to HSCs plated on plastic. However, when primary mouse HSCs were plated on a soft substrate (0.48kPa), expression of activation markers was reduced but ER stress was not prevented. In addition, treatment of HSCs with JNK inhibitors prevents the ER stress and reduces culture-induced activation of primary mouse HSC. This role for JNK was confirmed using JNK1 KO mice where decreased ER stress and activation were observed when isolated...
HSCs were plated. No effect on ER stress and activation was seen in HSCs from JNK2 KO mice.

**Conclusions:** ER stress induction is an early event during HSC activation *in vitro* and *in vivo*. Inhibition of ER stress by culturing cells in 3D spheroids inhibits HSC activation while cells seeded on soft substrates are less activated but ER stress is not affected. Finally, we show that this ER stress is JNK1 dependent. Together this strongly suggests that JNK1-dependent ER stress contributes to HSC activation, but is not sufficient to drive the activation process. We are currently investigating how the UPR is terminated and what the effects of a prolonged UPR would be.

**Disclosure of Interest:** None Declared
THE ROLE OF NECROPTOSIS IN CHRONIC CHOLESTASIS-INDUCED FIBROSIS

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Introduction: Progression to cirrhosis is one of the most nefarious clinical outcomes of chronic cholestatic liver diseases. Inhibition of necroptosis, a necrotic cell death pathway regulated by receptor-interacting protein 3 (RIP3), ameliorates hepatic fibrosis in methionine and choline-deficient diet-induced non-alcoholic steatohepatitis.

Aims: Here, we aimed to investigate the role of necroptosis in mediating fibrosis during cholestatic liver disease.

Material and Methods: Hallmarks of necroptosis were evaluated in liver biopsies of primary biliary cholangitis (PBC) patients. C57BL/6 wild-type and RIP3-deficient (RIP3⁻/⁻) mice were subjected to common bile duct ligation (BDL) for 3 and 14 days, to explore the role of necroptosis in both acute cholestasis and secondary biliary fibrosis. The functional link between RIP3 and the antioxidant response was investigated after BDL.

Results: Expression of RIP3 and its target phospho-mixed lineage kinase domain-like protein (p-MLKL) was found increased in liver samples of human PBC patients, coincident with thioflavin T labelling, suggesting activation of necroptosis. BDL resulted in progressive bile duct hyperplasia, multifocal necrosis, fibrosis and inflammation. Concomitantly, necroptosis was activated, as evidenced by increased RIP3 expression and activity, and sequestration of RIP3 and MLKL in the insoluble protein fraction of the liver. Remarkably, RIP3 deficiency blocked BDL-induced necroinflammation at 3 and 14 days post-BDL. Serum hepatic enzymes, fibrogenic liver gene expression and oxidative stress decreased in RIP3⁻/⁻ mice at 3 days after BDL. However, at 14 days, cholestasis aggravated and fibrosis was not ameliorated in RIP3⁻/⁻ mice. RIP3 deficiency further associated with increased hepatic expression of heme oxygenase-1 and accumulation of iron in BDL.
mice. Finally, TUNEL-positive cells and caspase-3/-7 activity increased 14 days after BDL in both WT and RIP3-/- mice, while remaining at basal levels at day 3, indicating that apoptosis is activated at late time-points in the BDL murine model, reflecting the peak of liver fibrosis.

**Conclusions:** Necroptosis is triggered in PBC patients and mediates hepatic necroinflammation in BDL-induced acute cholestasis. Targeting necroptosis may represent a therapeutic strategy for acute cholestasis, although complementary approaches may be required to control progression to fibrosis. (Supported by HMSP-ICT/0018/2011, SFRH/BD/91119/2012 and SFRH/BD/88213/2012 from FCT, Portugal.)

**Disclosure of Interest:** None Declared
ARTERIAL PRESSURE SUFFICES TO INCREASE LIVER STIFFNESS

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Introduction: Non-invasive measurement of liver stiffness (LS) has been established to screen for liver fibrosis. However, LS is also elevated in response to pressure-related conditions such as liver congestion.

Aims: This study was undertaken to learn more about the role of arterial pressure on LS.

Material and Methods: LS was measured using transient elastography (μFibroscan platform, Echosens, Paris) during single i.v. injections of catecholamines in anaesthetized rats with and without TAA-induced fibrosis. The effect of pressure-lowering drugs on LS was also studied. Pressures in the abdominal aorta, caval and portal vein were measured in real-time using the Powerlab device (AD Instruments, New Zealand).

Results: Baseline LS values in all rats (3.8±0.5 kPa, n=25) did not significantly differ from those in humans. Epinephrine and norepinephrine drastically increased mean arterial pressure (MAP) from 82 to 173 and 156 mmHg. Concomitantly, LS almost doubled from 4 to 8 kPa, while central venous pressure (CVP) remained unchanged. Likewise, portal pressure only showed a slight and delayed increase. In the TAA-induced fibrosis model, LS increased from 9.5±1.0 to 25.6±14.7 kPa upon epinephrine injection which could efficiently decreased by glycerolnitrate. We finally show a direct association in a physiological setting of elevated cardiac output in healthy volunteers. During continuous spinning at 200 W, MAP increased from 84 to 99 mmHg while LS significantly increased from 4.4 to 6.7 kPa.

Conclusions: In conclusion, our data show that arterial pressure suffices to increase LS. Moreover, lowering of MAP efficiently decreases LS in fibrotic livers that are mainly supplied by arterial blood.

Disclosure of Interest: None Declared
PREDICTION OF POST-OPERATIVE LIVER FAILURE IN CIRRHOTICS UNDERGOING SURGICAL RESECTION FOR HEPATOCELLULAR CARCINOMA: THE ROLE OF LIVER STIFFNESS MEASURED BY ACOUSTIC RADIATION FORCE IMPULSE AND THE PROPOSAL FOR A NEW MATHEMATICAL MODEL SCORE

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Introduction: Although the accurate selection of potential surgical patients, post-operative liver failure (PLF) accounts for 30-40% of complications after a liver resection for hepatocellular carcinoma in cirrhotics. A possible role of liver stiffness (LS) in the prediction of postoperative outcome has been suggested in some surgical series. However, the definition of PLF was different among the studies and patient selection wasn’t always careful. Therefore, a possible predictive power of LS in the identification of PLF is still unclear.

Aims: To evaluate a possible role of LS measured by Acoustic Radiation Force Impulse (ARFI) in the prediction of PLF after surgery for HCC in cirrhotics.

Material and Methods: From December 2010 to December 2014, 35 consecutive cirrhotics with HCC underwent liver resection in two reference Surgical Centers. Each of them was evaluated at our department for LS measurement (LSM) trough ARFI before surgery. Six patients were excluded from the study; 5 of them because the operative specimen excluded a cirrhotic evolution of the underlying disease and 1 patient because ARFI wasn’t reliable. Thereby, our population was composed by 29 consecutive pts.
**Results:** The majority were male (68.9 %) with a mean age of 69.4+9.9 y. The most frequent etiology was HCV infection (66 %). All patients were Child-Pugh A. Five pts (17%) were allocated in BCLC 0 and 14 pts (48 %) were allocated in BCLC A. At ROC curve, a cut off of 17.28 Kpa was identified as the best predictor of PLF in the overall population (Sensitivity: 83%; Specificity: 81%; AUC: 0.86). When we divided the population into 2 subgroups (BCLC 0+A: group A- n= 19; BCLC B: group B – n= 10) we observed as in “very early and early stage” patients, LSM was very accurate in predicting PLF (AUC: 0.99 vs 0.79 in group B; p=0.06; Fig.1). On this basis, we restricted our analysis to group A with the aim to explore other possible variables related to the onset of PLF. At multivariate analysis, only the LSM and the spleen size were confirmed as independent predictors of PLF. We included these two variables into a mathematical model (MPPLF: model for prediction of post-operative liver failure): [(0.09*spleen diameter) + (0.03*LS)]. A cut-off of 2.04 was able to identify an episode of PLF with a sensitivity and specificity of 100%.

**Conclusions:** LSM is an important diagnostic tool in selection of potential surgical patients BCLC 0 or A, especially when included with spleen size in our mathematical predictive model.

**Figure:**

![ROC curve](image.png)

**Disclosure of Interest:** None Declared
Screen 2: YI-MP-I85

PLATELET SPECIFIC JUNCTIONAL ADHESION MOLECULE-A DEFICIENCY ATTENUATES EXPERIMENTAL LIVER FIBROSIS IN MICE

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Introduction: Apart from their function in haemostasis, platelets play a pivotal role in several inflammatory diseases including liver fibrosis. During chronic liver injury platelets secrete different mediators that can drive both pro- or anti-fibrotic and inflammatory pathways. Recently, the platelets’ specific deletion of junctional adhesion molecule-A (JAM-A) was associated with a hyperactive state of platelets.

Aims: Here we aimed to investigate the role of these hyperactive platelets in liver fibrosis in order to shed light on the complex role of platelets during chronic liver injury.

Material and Methods: Liver fibrosis was induced in PF4 Cre⁺ JAM-A flox/flox and PF4 Cre⁻ JAM-A flox/flox control mice by intraperitoneal injections of carbon tetrachloride (CCl₄) or thioacetamide (TAA) over 6 weeks. Liver fibrosis was quantified by Sirius red staining and hydroxyproline assays. Stellate cell activation was analyzed by α-SMA staining. mRNA expression of genes relevant in fibrosis was assessed in mice as well as during in vitro studies of GRX cells stimulated with thrombin supplemented platelet-rich plasma obtained from both mouse strains using qPCR techniques. Infiltration of immune cells was determined by immunofluorescence staining. Minding the recent discovery that platelets affect inflammatory responses by influencing the differentiation of T cell subsets e.g. Th17 cells, mRNA expression of CD4+ T cell differentiation associated transcription factors was analyzed by qPCR.

Results: In both models, platelet specific deletion of JAM-A resulted in ameliorated liver fibrosis compared to control mice. Moreover, the absence of JAM-A in platelets was associated with reduced stellate cell activation and less collagen mRNA expression in liver tissue and platelet-rich plasma activated stellate cells. The number of infiltrating
T cells and neutrophils was reduced and interestingly, the expression of RUNX as a Th17 associated transcription factor was decreased in livers that lack platelet specific JAM-A.

**Conclusions:** Here we show that platelet specific JAM-A deletion reduced liver fibrosis accompanied by decreased stellate cell activation. Furthermore, JAM-A deficiency of platelets is associated with an altered intrahepatic immune cell infiltrate with a shift in T cell subdifferentation on the transcriptional level. These results highlight an important immunomodulatory role of platelets during liver fibrogenesis.

**Figure:**

**Disclosure of Interest:** None Declared
DIVERSE ROLES OF AMINE OXIDASES IN LIVER FIBROSIS

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Introduction: Lysyl oxidase (LOX) and lysyl oxidase-like (LOXL) enzymes are amine oxidases (AO) which catalyse covalent cross-linking of side chains of collagen and elastin and thus can further mature scar. There is increasing evidence that LOX proteins (LOX, LOXL1-4) also have a diverse range of additional functions, including stimulating cell migration and invasion. We have previously reported that another AO vascular adhesion protein-1 (VAP-1) is drive inflammation and fibrogenesis during hepatic injury.

Aims: Investigate the role of amine oxidase in hepatic fibrogenesis.

Material and Methods: Expression and regulation of amine oxidases were investigated in human liver tissue and murine models of hepatic injury.

Results: VAP-1 deficient mice have reduced elastin, LOX and LOXL2 mRNA expression following 8 weeks of CCl4 treatment when compared to wild type animals. TGFβ1 is one of the most potent pro-fibrotic growth factors and has previously been reported to stimulate the production of collagen and elastin in fibroblasts. TGFβ1 mRNA was markedly reduced in VAP-1 knockout animals following 8 weeks of injury when compared to wild type control animals. Furthermore, modulation of VAP-1 in the human stellate cell line LX-2 revealed that overexpression of VAP-1 resulted in a 3.6 fold induction of TGFβ1 mRNA and silencing of VAP-1 by siRNA resulted in a 5 fold decrease in TGFβ1 mRNA. TGFβ1 has also been reported to induce the expression of lysyl oxidases in fibroblasts. LOX and LOXL expression have been reported in human hepatocellular carcinoma and Wilson’s Disease but there remains a paucity of data for expression and function in a wider context in chronic end stage liver disease. We analysed the relative expression of amine oxidases in chronic liver diseased liver tissue by qRT-PCR and report that the expression of VAP-1, LOX and LOXL-1 through to -4 is upregulated in end stage disease tissue with the exception of autoimmune hepatitis. Furthermore, analysis of isolated human hepatic cells reveals that the hepatic stellate cells (HSC) and activated liver myofibroblasts (aLMF) are the major source of LOXs in the diseased liver.
Conclusions: VAP-1 and LOXs are up-regulated in fibrotic liver disease. VAP-1-dependent TGFβ1 modulation suggests a mechanism by which VAP-1 may drive fibrogenesis. Upregulation of ECM protein synthesis as well as upregulating enzymes involved in cross-linking ECM, in combination with increased inflammatory infiltrate recruitment, would perpetuate fibrosis and further mature the scar.

Disclosure of Interest: None Declared
**ACTIVATION OF CEPBA BY OLGONUCLEOTIDE SARNA THERAPY IN PROGRESSIVE LIVER FAILURE REVERSES LIVER FIBROSIS AND PROMOTES LIVER REGENERATION**

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**Introduction:** Chronic liver disease is a growing epidemic worldwide responsible for progressive liver fibrosis and liver failure. CCAAT/ enhancer binding protein alpha (C/EBP\(\alpha\)) is one of the master regulators in the liver for normal differentiation and metabolic function. Its attenuation is frequently observed in liver disease.

**Aims:** We have evaluated three different models of chronic liver disease to determine if therapeutic activation of CEBPA, achieved by intravenous delivery of a small activating RNA (saRNA) to CEBPA encapsulated in NOV340 SMARTICLES\(^5\) (MTL-CEBPA), would reverse liver failure.

**Results:** Non-alcoholic steatohepatitis was induced in C57BL/6 mice with methionine choline deficient diet (MCD). Upon administration of MTL-CEBPA (0.3, 1 and 3mg/kg) we observed a significant 55% reduction in staining of alpha smooth muscle actin compared to control. In addition we saw significant reduction of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) to near normal levels and significant 70% reduction in liver triglyceride and hepatic lipid accumulation at all dose range. To induce hepatic fibrosis, wild-type Sprague Dawley (SD) rats were treated for 10 weeks with Carbon tetrachloride (CCL\(_4\)). Injury to the liver were confirmed by Sirius red and Masson’s trichrome staining. AST, ALT and hydroxyproline showed significant
reversal to near normal levels after 2 weeks of MTL-CEBPA treatment (4 doses of 3.0 mg/kg). Moreover, mortality significantly decreased (11% in MTL-CEBPA treated groups vs 44% in control).

An acute liver failure model was induced in SD rats subjected to 350mg/kg of Thioacetamide (TAA) intraperitoneally. Similar to the above models a significant improvement in all liver parameters measured including ALT, AST and bilirubin were observed following a single dose of MTL-CEBPA injection.

**Conclusions:** These studies demonstrated the crucial role of C/EBPα in maintaining normal liver function and highlight the potential of using MTL-CEBPA to activate CEBPA as a treatment of liver fibrosis and other liver disease.

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LIVER FIBROSIS IN CORRELATIONS WITH CD4/CD8 AND PLATELET COUNT IN PATIENTS WITH CHRONIC HEPATITIS DELTA

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Introduction: Platelets participate to the recruitment of cells of the innate and adaptative immune response implicated in liver fibrosis.

Material and Methods: A cross-sectional study was performed among the 152 patients with HDV – infection (mean age: 41 ± 4.6 years, 91/61 – 59.86%>men). Inclusion criteria: presence of HDV total antibodies, positive HDV RNA. Exclusion criteria: decompensated liver fibrosis, neoplastic disease, recent episodes of gastrointestinal disease. The CD4, CD8 was appreciated used flow cytometry. Liver fibrosis was assessed by transient elastography or by Fibrotest.

Results: Positive HDV RNA was found in 132/152 (86.8%), positive HBV DNA in 58/152 (38.1%). HDV- infected patients with F3 – F4 liver fibrosis had lower CD4/CD8 ratios (1.4) and the mean platelet count – 142.33 ± 46.51, with F0-F2 fibrosis had CD4/CD8 – 2.9 (p = 0.05) and platelet count – 190.67 ± 38.45 (p <0.05). We identified correlation: HDV viremia level and CD4 (r = 0.56, p < 0.05) and CD8 (r = 0.53, p < 0.01), platelet count and CD8 (r = 0.61, p <0.01), CD4/CD8 ratio and liver fibrosis (r = -0.62, p = 0.01).

Conclusions: This study emphasis the relevance of CD4/CD8 and thrombocytopenia in the liver fibrosis for patients with chronic hepatitis delta.

Disclosure of Interest: None Declared
Screen 6: MP-104

FIBROSCAN VERSUS LIVER BIOPSY IN THE EVALUATION OF RESPONSE AMONG THE EGYPTIAN HCV INFECTED PATIENTS TO TREATMENT

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Introduction: Hepatitis C virus (HCV) infection usually progress to chronic infection with subsequent cirrhosis and cancer. Therapies aim to eradicate the virus and prevent further progression. Interferon is claimed to have anti-fibrotic effect. Histopathology is the gold standard in diagnosis and grading of hepatic fibrosis, but Transient elastogram (Fibroscan) can be used as alternative noninvasive modality.

Aims: This prospective study aimed to evaluate the accuracy of fibroscan in diagnosis of liver fibrosis, and assess the effect of antiviral therapy on fibrosis stages in HCV patients.

Material and Methods: The study was conducted from September 2012 to December 2014 as a project funded by Science and Technology Development Fund, Egypt, Grant No. 3448. It included 498 patients; 150 HCV cirrhotic patients as control, and 348 HCV naive patients grouped according to their liver biopsy into; mild (groupI) and moderate (groupII) fibrosis. They were examined using fibroscan (Echosens, Paris, France, device 502, M probe) before, 12, 24, and 48 weeks of therapy, with 300 patients (150 patient in each group) completed follow up regardless their response. The results of fibroscan were compared to each other and to liver biopsy.

Results: Fibroscan can diagnose F1 at 6 k Pascal with 26% sensitivity, 8% specificity, AUC=0.037; F2 at level of 7 K Pascal with 84.6% sensitivity, 71.3% specificity, AUC = 0.692 and F3 at 9.5 k Pascal with 96% sensitivity, 97% specificity, AUC = 0.997. The fibrosis results had regressed significantly after 48 weeks of starting therapy of both patients’ groups (p<0.05). When categorized by response to therapy, Responders showed significant decline in their fibroscan scores compared to non-responders of same fibrosis degree.

Conclusions: Fibroscan correlated to histopathology in moderate (F2-F3), but not mild (F1) fibrosis. The degree of fibrosis regresses significantly in HCV responders on anti-
viral INF based therapy. Besides its accuracy as noninvasive device in detecting degree of fibrosis, fibroscan can be very useful in assessment of degree of fibrosis during and after therapy.

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FISHING FOR NOVEL TARGETS OF LIVER FIBROSIS 
BY USING DANIO RERIO MODEL

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Introduction: Liver fibrosis results from the excessive accumulation of extracellular matrix (ECM) proteins that may distort normal tissue architecture and lead to organ dysfunction. To date, transplantation is the only curative therapy available for end stage fibrotic diseases, however, associated with a high risk of complications. Therefore there is still an unmet need for the effective and safe treatment of liver fibrosis. The progress in developing of anti-fibrotic therapies is affected by the lack of specific molecular targets for anti-fibrotic therapy and biomarkers that could allow accurate assessment of fibrosis progression or reversal.

Aims: Recently, next-generation sequencing is a powerful technique allowing the identification of novel genes, non-coding RNAs and enhancers related to pathophysiological condition both in model organisms and in clinics. However, genomics-based data require functional characterization and validation in a biological model. The goal of this study is to use zebrafish model of liver injury to study the role of novel targets of liver fibrosis.

Material and Methods: Zebrafish has emerged as a valuable organism to study liver development and disease. The liver of zebrafish fully develops by 72 hours post-fertilization and contains the same main cell type as can be found in mammals, such as hepatocytes, biliary cells, endothelial cells (EC) and hepatic stellate cells (HSC). To study potential molecular targets of liver fibrosis we approach CRISPR/Cas9-based genome editing. The translucence of zebrafish embryos and larvae enables suitable in vivo imaging analysis, therefore we establish a thioacetamide (TAA)-inducible model of liver injury in transgenic zebrafish lines, specifically expressing fluorescent proteins in HSC, EC and hepatocytes.
This allows us to investigate cell behaviours during liver injury and regeneration as well as to obtain specific liver cell types by fluorescence-activated cell sorting (FACS) for studying the regulatory networks driven by candidate genes.

**Results:** Results from this study will provide valuable information on the function of potential molecular targets of liver fibrosis and will contribute to the identification of novel therapeutic strategies.

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**Disclosure of Interest:** None Declared
Screen 3: MP-III

VALIDATION OF ELF TEST SCORE FOR HEPATIC FIBROSIS EVALUATION IN TURKISH CHRONIC LIVER DISEASE PATIENTS

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Introduction: Determining the severity of liver fibrosis without liver biopsy has been getting higher priority in order to clarify prognosis or response of treatment in patients with chronic liver disease. The Enhanced Liver Fibrosis (ELF) test is a simple blood test to create an ELF score by combining three markers: hyaluronic acid (HA), amino-terminal propeptide of type III procollagen (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1).

Aims: Our aim was to validate the ELF test in a group of biopsy proven chronic hepatitis patients in a tertiary care university hospital in Turkey.

Material and Methods: Patients with chronic Hepatitis B (CHB), chronic hepatitis C (CHC), and autoimmune hepatitis (AH) who have liver biopsies in 6 to 12 months were recruited and serum samples for ELF test were collected. Liver biopsies of these patients have already been used for digital imaging analysis (DIA) in a previous study for quantification of type III procollagen in liver biopsy samples. The validation of ELF test score solely and, its components were done by Ishak and Knodell fibrosis scores. DIA quantification and serum procollagen type III levels were evaluated for any correlation.

Results: Fifty four patients (Female 26, 35 CHB, 17 CHC, 2 AH) and 37 healthy volunteers (Female 19) were enrolled into the study. The accuracy of the ELF test for distinguishing healthy and liver fibrosis was very good (AUROC: 0.910, sensitivity 84%, specificity 89%), HA which is a component of the ELF test (AUROC: 0.908, sensitivity 75%, specificity 94.4%) found to be as strong as ELF test. For detection of significant fibrosis (Ishak ≥F2, Knodell ≥F1) cut off value of 8.41 (AUROC: 0.90, sensitivity 87%, specificity 82%), and for detection of severe fibrosis (Ishak ≥F4; Knodell ≥F3) cut-off value of 9.47 (AUROC: 0.79-0.81, sensitivity 75-65%, specificity 75-84%) were found
to be appropriate for our population. DIA type III procollagen values were moderately correlated with the serum type III procollagen values (rs: 0.441, p = 0.001).

**Conclusions:** The ELF test is a promising technique to identify patients with liver fibrosis, especially for the clarification of significant and/or severe fibrosis in our population. However, cut off levels for the significant and/or severe fibrosis should be redetermined for our population. Serum procollagen type III levels are probably effected by another fibrotic tissues in the body.

**Disclosure of Interest:** None Declared
ANTIFIBROGENIC EFFECT OF STATINS USED FOR TREATMENT OF NON-ALCOHOLIC STEATOHEPATITIS (NASH)

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Introduction: NASH is considered the extreme appearance of NAFLD and an important risk factor for liver fibrosis. Although preliminary data indicates that statins may be beneficial when given for NASH treatment, recent reports are controversial.

Aims: To evaluate if statins independently influence the evolution of fibrosis accompanying NASH using the FibroTest, SteatoTest and NashTest scales of FibroMax.

Material and Methods: 80 patients with NASH and metabolic syndrome were followed-up for a period of 2 years. Exclusion criteria were as follows: a series of drugs, regular alcohol intake>60g/day, genetic metabolic disorders or impaired intestinal absorption (celiac disease). Steatosis, fibrosis and NASH were quantified by using the FibroMax scales at baseline and after two years of statin treatment. Patients were randomized in two groups: the active group of 40 patients receiving low-dose hydrophilic statin (rosuvastatin 5mg/day) and the witness group of 40 patients, matched by age, gender and sex, receiving placebo.

Results: 98% of subjects fulfilled the follow-up period. The FibroMax staging at baseline showed the following results in the active group: S1 – 27%, S2 – 43% and S3 – 30%; F1 – 52%, F2 – 28%, F3 – 11% and F4 – 9% of patients, respectively N1 – 28% and N2 – 72%. The staging according to FibroTest, SteatoTest and NashTest was similar in placebo group. After 2 years of low-dose hydrophilic statin, the mean ALT level from active group decreased from 64.35 IU/L to 32.19IU/L, p < 0.05 (ss); in the witness group no significant ALT decrease was noticed (66.15IU/L to 59.22IU/L, p>0.5). The FibroMax showed an important improvement of steatosis and fibrosis in active group, compared with the witness group. After two years of statins, our active group was stratified as follows: S0 – 23%, S1 – 44%, S2 – 29%, respectively S3 – 4% of patients, respectively F0 – 33%, F1 – 36%, F2 – 26%, F3 – 5%; F4 – 0% of patients. NashTest also proved a positive evolution
under statin treatment, compared with placebo (N0 – 35%, N1-37% respectively N2-28% , p>0.001, ss) After adjusting for age, BMI, diabetes, LDL-cholesterol and triglyceride levels, statin therapy showed a significant correlation with the steatosis, fibrosis and NASH stages improvement in the active group (r=0.92, r=0.87, respectively r=0.95, p<0.005, ss).

Conclusions: In our study, statins proved to be safe and efficient for the treatment of NASH, but larger clinical studies are needed to further demonstrate this beneficial effect.

Disclosure of Interest: None Declared
DOES ELEVATED SINUSOIDAL PRESSURE CAUSES LIVER CIRRHOSIS? THE SINUSOIDAL PRESSURE HYPOTHESIS AND ROLE OF ARTERIALIZATION

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Introduction: All chronic liver diseases ultimately lead to liver cirrhosis which is a major health problem worldwide. The underlying molecular mechanisms are still poorly understood and no efficient treatment strategies are available. Moreover, several common observations cannot be explained such as a) the almost uniform response of cirrhosis to the various etiologies b) the typical macroscopic features of cirrhosis such as large fibrous septa spanning over several centimeters through the organ and c) reversal of fibrosis with the so called ‘point of no return’.

Aims: Here, the sinusoidal pressure hypothesis (SPH) is introduced and discussed in detail. So far, pressure changes have been mainly seen as a consequence of cirrhosis but not as cause.

Material and Methods: Liver stiffness measurements in patients with various liver disease and in various animal models using transient elastography and μFibroscan (both Echosens, Paris) have been used. In additon, pressur was measured directly in animal models in the abdominal aorta, caval and portal vein in real-time using the Powerlab device (AD Instruments, New Zealand).

Results: According to SPH, sinusoidal pressure (SP) is a major upstream event in initiating cirrhosis. SPH has been mainly derived from recent clinical and experimental findings on liver stiffness (LS) that not only increases in response to fibrosis but also pressure changes such as liver congestion. In line with SPH, all potential causes of cirrhosis ultimately lead to an elevated SP whether of inflammatory or non-inflammatory origin. Fibrosis progression is determined by the degree and time of elevated SP with the cirrhotic matrix eventually matching SP. A SP elevation over 12 mmHg and over 4 weeks seems to be a critical threshold. Arterialization of the cirrhotic liver represents the final self-perpetuating key event finally exposing the low-pressure organ liver to pathologically high pressures and
defining the point of no return. At the cellular level, SP is the actual driving force for the production of extracellular matrix by stretching of perisinusoidal cells e.g. hepatic stellate cells via cellular and intercellular mechano-signaling.

Conclusions: SPH is able to explain common clinical observations such as macroscopic architecture of the cirrhotic liver and the uniform fibrotic response to various etiologies. The novel concept will hopefully stimulate the search for novel treatment strategies.

Figure:

Disclosure of Interest: None Declared
DIRECTED DIFFERENTIATION OF IPS CELLS TO HEPATIC STELLATE CELLS

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Introduction: In a healthy liver, hepatic stellate cells (HSC) are responsible for extracellular matrix homeostasis and accumulating vitamin A in lipid droplets. The lack of renewable sources and homogeneous cultures of quiescent HSC has prevented its application in co-culture (with hepatocytes) in vitro systems to perform fibrogenic and toxicological studies.

Aims: The current study aimed to develop a protocol for generating HSC derived from human induced pluripotent stem cells (iPSC).

Material and Methods: The differentiation protocol developed is based on embryonic/fetal liver development. The differentiation was monitored by assessing gene expression and FACS of the intermediate and final differentiation population markers. IPSC-derived HSC were characterized by qPCR, immunocytochemistry and transcriptomic analysis. Differentiated cells were functionally characterized.

Results: The differentiation led to subsequently populations mimicking fetal liver development such as multipotent mesoderm population, liver mesenchymal cells, mesothelial cells and HSC. The intermediate populations were characterized by assessing gene expression of key HSC markers. The final HSC–like population was enriched in PDGFRβ cells (50-70%) and expressed HSC markers at a gene (αSMA, Col1α1, PDGFRβ and PPARγ) and at protein level (PDGFRβ, ACTA2, NGF, vimentin, N-CAM) as assessed by immunocytochemistry. Comparative whole genome transcriptomic analysis showed that IPSC-derived cells showed an intermediate phenotype between primary human
activated and quiescent HSCs. IPSC-derived cells were able to expand for 3 doublings in 2-D monocultures and could be aggregated in spheroids with human hepatocytes. A functionally analysis of the differentiated cells showed that iPS-derived HSC-like cells (1) respond to transformig growth factor β and lipopolysaccharide expressing pro-fibrogenic, pro-inflammatory and activation markers; (2) increase their migration capacity after PDGF or FBS incubation and finally (3) possess the capacity to incorporate retinyl esters into lipid droplets.

**Conclusions:** We have developed for the first time a protocol to differentiate iPSC to HSC-like cells. iPSC progeny is enriched in PDGFRβ-positive cells and express typical HSC markers. IPSC-derived HSC-like cells may have great potential to perform fibrogenic and toxicological assays.

**Disclosure of Interest:** None Declared
INTEGRIN ALPHA II IN REGULATION OF MYOFIBROBLASTS PHENOTYPE: IMPLICATION FOR FIBROTIC DISEASES

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Introduction: Liver fibrosis, characterized by the excessive accumulation of extracellular matrix produced by proliferative and differentiated hepatic stellate cells (HSCs) or myofibroblasts, is the growing cause of mortality worldwide. Thus, understanding of the factors that induce HSCs differentiation is paramount to prevent the fibrotic process. Mechanical stress derived from the integrin-mediated interaction between ECM and the cytoskeleton promotes HSCs differentiation.

Aims: In this study, we aimed to explore the significance of Integrin alpha 11 (ITGA11) in HSCs during liver fibrosis.

Material and Methods: ITGA11 expression and its correlation with fibrotic parameters were examined in fibrotic mouse livers, cirrhotic human livers and in TGFβ-activated human HSCs. To elucidate ITGA11 role in HSCs, stable ITGA11 knock-down (ITGA11-KD) cells were generated using ITGA11-shRNA plasmid. Changes in HSCs morphology and fibrotic parameters were studied in ITGA11-KD HSCs using AFM, immunostainings, PCR and profiler human fibrosis array. Furthermore, to assess its functional role, 3D-collagen gel contraction and wound healing assays were performed.

Results: ITGA11 expression was highly induced and correlated with increasing fibrosis in-vivo in fibrotic mouse models. ITGA11 mRNA expression was significantly (p<0.04)
increased at stage F3 or F4 (severe fibrosis) as compared to stage F0 or F1 (mild fibrosis) in NASH patients. ITGA11 expression was found to be co-localized with α-SMA positive HSCs in both mouse and human cirrhatic livers. Furthermore, low expression of ITGA11 was found in normal mouse organs and healthy human organs tissue microarray. In-vitro, ITGA11 expression levels were highly up-regulated in TGFβ-activated human HSCs while remained undetected in human hepatocytes and monocytes. Stable ITGA11-KD HSCs demonstrated drastic reduction in TGFβ-induced fibrotic parameters. Strikingly, in profiler fibrosis array, ITGA11-KD HSCs showed a significant reduction in 19 fibrosis-related genes. In the functional assays, ITGA11-KD resulted in attenuated wound healing, reduced adhesion and impaired collagen contractility. Eventually, ITGA11 expression was found to be significantly up-regulated in different organ fibrosis in human patients suggesting ITGA11 regulates fibrosis development in different organs.

**Conclusions:** These findings highlights the therapeutic significance of ITGA11 in organ fibrosis and development of ITGA11 antagonizing strategies could lead to development of novel therapies against fibrotic diseases.

**Disclosure of Interest:** None Declared
SIMVASTATIN IMPEDES THE ENDOTOXIN-INDUCED AGGRAVATION OF HEPATIC MICROVASCULAR DYSFUNCTION IN TWO ANIMAL MODELS OF LIVER CIRRHOSIS

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Introduction: In liver cirrhosis, portal hypertension and variceal bleeding are associated and aggravated by bacterial infections; however the influence of bacterial infections on hepatic microcirculation in cirrhotic livers remains unknown. Statins have shown beneficial effects ameliorating liver endothelial dysfunction in cirrhosis.

Aims: We aimed at characterizing the effects of simvastatin on liver microvascular dysfunction in cirrhotic rats with endotoxemia.

Material and Methods: Lipopolysaccharide (LPS; 1mg/kg) or saline was given to: (1) CCl4 and BDL-cirrhotic rats treated with placebo, or (2) CCl4 and BDL-cirrhotic rats treated with simvastatin (25 mg/kg/24h, orally) 3 days prior. Hepatic and systemic Hemodynamic parameters followed by Portal Perfusion Pressure (PPP) and microvascular functionality were assessed. Underlying molecular mechanisms (liver fibrosis, HSC activation, inflammation, oxidative stress, NO release) were investigated in the CCl4 cirrhotic model

Results: Simvastatin pre-treated LPS cirrhotic rats had lower PP (10.8±0.8 vs.14.1±1 mmHg; -24% in CCl4 & 11.3±0.8 vs.14.3±0.8mmHg; -26% in BDL) and HVR (0.73±0.1 vs. 1.0±0.2 mmHg/mL·min-1; -31% in CCl4 & 0.63±0.2 vs. 0.7±0.7mmHg/mL·min-1; -16% in BDL), and improved MAP (112±10 vs. 94±5 mmHg; +15% in CCl4 & 71±10 vs. 65±6 mmHg;+9% in BDL) without significant changes in PBF, in comparison to vehicle-treated LPS cirrhotic rats. Simvastatin pretreatment prevented the LPS-induced increase in PPP (7.3±0.3 vs. 12.4±0.8 mmHg; -41% in CCl4 and 10.2±0.2 vs.11.3±0.8 mmHg; -11% in BDL) and significantly ameliorated the hepatic microvascular dysfunction in response to Ach. In the CCl4 group, beneficial effects were associated with significant
reductions in pro-inflammatory markers and HSC activation (iNOS -17%, ICAM -33%, CD68 -74%, TLR4 -30%, αSMA -51% at protein levels; and iNOS -44%, IL-6 -60%, SMA -18% at mRNA levels) and increments in anti-inflammatory marker (IL-10 +45%) and eNOS phosphorylation +21%. Simvastatin reduced reactive oxygen species (O2- -60%, ONOO--52%) in comparison to vehicle-treated LPS cirrhotic rats.

**Conclusions:** Our study demonstrates that simvastatin pre-treatment prevents endotoxemia-induced hepatic microvascular dysfunction in cirrhosis. This effect is achieved by ameliorating sinusoidal NO availability, hepatic inflammation and oxidative stress.

**Disclosure of Interest:** None Declared
ACCURACY OF TRANSIENT ELASTOGRAPHY IN PREDICTING HISTOLOGICAL FIBROSIS SEVERITY IN TREATED AUTOIMMUNE HEPATITIS

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Introduction: Transient elastography (Fibroscan) in assessment of severity of liver fibrosis has been validated against liver biopsy in chronic viral hepatitis and in NAFLD. There are few data in autoimmune hepatitis (AIH). In four preliminary studies, most patients had recently started treatment and thus, still had active disease.

Aims: To assess accuracy of Fibroscan in predicting histological fibrosis severity in patients who had achieved normal biochemical remission and were undergoing follow-up liver biopsy to confirm histological remission.

Material and Methods: Same-day Fibroscan (Echosens, with M or XL probe as needed) and liver biopsy were performed in 32 fasted patients with AIH (1999 International Group criteria; 25 female, age 56 (17-78) years who had achieved normal serum ALT and globulins after 2.7 (2.1-24.9) years treatment. We assessed how accurately the liver stiffness evaluation (LSE) score on Fibroscan could predict Ishak fibrosis stage on biopsy (assessed independently by AKD). The ALT was 21 (9-99), normal value <33. 89% patients had normal serum ALT on the day. Of the 36 Fibroscan’s carried out, 27 were valid (≥10 liver stiffness measurements, interquartile range (IQR)/median of <0.30, success rate ≥60%).
**Results:** Results are summarised in the table below:

**Table 1: Accuracy of Fibroscan in Prediction of Liver Fibrosis**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Ishak Fibrosis Stage (n)</th>
<th>AUROC</th>
<th>Fibroscan cut-off score</th>
<th>Sens. (%)</th>
<th>Spec. (%)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n=36)</td>
<td>5 or 6 (n=5)</td>
<td>0.78</td>
<td>11.0</td>
<td>0.60</td>
<td>0.80</td>
<td>0.33</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>4-6 (n=11)</td>
<td>0.78</td>
<td>11.0</td>
<td>0.55</td>
<td>0.88</td>
<td>0.67</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>3-6 (n=21)</td>
<td>0.62</td>
<td>7.0</td>
<td>0.52</td>
<td>0.66</td>
<td>0.69</td>
<td>0.50</td>
</tr>
<tr>
<td>Valid scans (n=27)</td>
<td>5 or 6 (n=3)</td>
<td>0.97</td>
<td>11.0</td>
<td>1.00</td>
<td>0.83</td>
<td>0.43</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>4-6 (n=6)</td>
<td>0.90</td>
<td>11.0</td>
<td>0.83</td>
<td>0.90</td>
<td>0.71</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>3-6 (n=15)</td>
<td>0.72</td>
<td>7.0</td>
<td>0.46</td>
<td>0.83</td>
<td>0.78</td>
<td>0.55</td>
</tr>
</tbody>
</table>

**Conclusions:** Fibroscan showed good accuracy in excluding, but lower accuracy in predicting Ishak fibrosis stage of 4 or more. Accuracy was improved if a valid scan was obtained. Fibroscan was less accurate in predicting lower fibrosis stages.

**Disclosure of Interest:** None Declared
COFFEE CONSUMPTION IS PROTECTIVE OF LIVER STIFFNESS IN THE GENERAL POPULATION: THE ROTTERDAM STUDY

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Introduction: Several studies have shown a potential protective effect of coffee on fibrosis and/or cirrhosis in patients with established liver disease. However, it is not known if this is also true in a preclinical setting.

Aims: Therefore we aimed to study the effect of coffee consumption on liver fibrosis, assessed by transient elastography (TE), in a large well-characterized population.

Material and Methods: The Rotterdam study is an ongoing prospective population-based cohort study of healthy inhabitants of the suburb Ommoord, started in 1990. From 2009 onwards, all participants aged >=45 underwent TE and completed a validated 389-item food frequency questionnaires (FFQ) including detailed information on coffee consumption. Correlation, linear-and logistic regression analyses were used to study the association between coffee consumption and liver stiffness measurements (LSM). LSM>=8.0kPa was considered clinically relevant fibrosis and secondary causes of increased LSM (e.g. viral hepatitis, congestive heart failure) were excluded. Coffee was categorized into no, low (<=2), modest (3-4) or high (>=5) consumption (cups/day).

Results: We included 2424 participants (age 66.5±7.4) of which 125 (5.2%) had LSM>=8.0kPa. No coffee consumption was reported by 166 (6.8%) participants, while 698 (28.8%) reported low, 961 (9.6%) modest and 599 (24.7%) high coffee consumption. Prevalence of LSM>=8.0kPa in these categories was 7.8%, 6.9%, 4.5% and 3.5% respectively. Also, risk of fibrosis decreased with increasing coffee consumption (OR_low=0.9 CI 0.5-1.6, OR_modest=0.5 CI 0.3-1.1, OR_high=0.4 CI 0.2-0.9, P_trend=0.014). This inverse relation between coffee intake and LSM was also observed in linear regression...
(Beta_{modest}=-0.04 \; CI-0.03;-0.01, \; Beta_{high}=-0.03 \; CI-0.07; 0.001, \; P_{trend}<0.001) \text{ and even enhanced after adjusting for age, sex, ALT, steatosis, BMI, HOMA-IR, smoking and excessive alcohol, total energy and tea intake (Beta}_{modest}=-0.065 \; CI-0.118;-0.012, \; Beta}_{high}=-0.060 \; CI-0.116;-0.003, \; P_{trend}=0.006). Additional adjustment for milk and sugar use in coffee did not affect this association.

**Conclusions:** Great coffee consumption is independently associated with lower liver stiffness measurements in the general population. As one of the most popular beverages worldwide it is very interesting that the protective effect of coffee appears to already occur in the preclinical state. More prospective studies confirming this association are needed to help develop preventive strategies for a healthy liver.

**Figure:**

![Multivariate linear regression analyses testing the association between coffee consumption and (log-transformed) LSM.](image)

**Disclosure of Interest:** None Declared
ALTERATIONS IN CATIONIC AMINO ACID TRANSPORTERS AND OXIDATIVE STRESS IN THE DEVELOPMENT OF NON-ALCOHOLIC STEATOHEPATITIS

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Introduction: The solute carrier family-7, SLC7, is divided into two subgroups, the cationic amino acid transporters, CATs, (SLC7A1–4) and hetero(di)meric amino acid transporters, HATs, (SLC7A5–11). Asymmetric-dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, is transported by CATs. The genetically obese mice develop non-alcoholic steatohepatitis (NASH) by iron-supplemented diet.

Aims: Using an experimental model of NASH induced by methionine/choline-deficient (MCD) diet, this study investigated the oxidative stress, the iron levels and the potential changes in CAT-1, CAT-2A and CAT-2B.

Material and Methods: NASH was induced in Male Wistar rats by 8-week feeding with MCD diet. Serum and hepatic biopsies, collected at 2, 4 and 8 weeks, were used. Serum hepatic enzymes (AST, ALT), iron, uric acid and ADMA were evaluated. Tissue total lipids, lipid peroxides (TBARS), glutathione and reactive oxygen species (ROS) were also quantified. Hepatic biopsies were used for the analysis of ADMA and its transporters as liver mRNA expression of CAT-1, CAT-2A and CAT-2B.

Results: Lower serum levels of uric acid and higher levels of iron were found in MCD rats compared with control group. The hepatic decrease in glutathione content was concomitant with an increase in TBARS and ROS levels. A time-dependent increase in serum AST and ALT and in hepatic total lipids and a decrease in serum and tissue ADMA were found in MCD group. Higher mRNA expression of CAT-1 and lower in CAT-2A and CAT-2B were found using MCD diet; a positive correlation was found between CAT-1 and TBARS levels as well as a negative correlation was detected comparing CAT-2A and CAT-2B and TBARS content.
**Conclusions:** The spontaneous development to severe NASH observed in MCD rats is associated with increased serum iron and oxidative stress; changes in both ADMA levels and its transporters represent an innovative factors involved in the onset and the progression of hepatic alteration associated with MCD-diet-induced NASH.

**Disclosure of Interest:** None Declared
EPITHELIAL AND MESENCHYMAL MOLECULAR PROFILES IN AN EXPERIMENTAL MICROSURGICAL MODEL OF CHOLESTASIS – THEIR RELATIONS TO EPITHELIAL-MESENCHYMAL TRANSITION AND HEPATIC FIBROSIS

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Introduction: Human chronic extrahepatic biliary cholestasis produces a variable proliferation of epithelial biliary cells. Additionally, hepatic fibrosis and prominent microadenomatous biliary transformation of the liver can occur in the most severe cases.

Aims: We sought to determine histopathological changes triggered by cholestasis in an experimental model.

Material and Methods: We developed an experimental chronic model of microsurgical cholestasis in rat by performing a complete extrahepatic ligation of the four lobar hepatic ducts. Eight weeks later, animals were sacrificed and both caudal and medium hepatic lobes were removed and fixed in formaldehyde at 4%. Immunohistochemical expression of keratin 19 (K19), vimentin (Vim), collagen type I (Col I), collagen type III (Col III), and albumin (Alb) and densitometric quantifications were determined.

Results: Lobular hepatic pattern was not recognized, finding evident adenomatous biliary intrahepatic changes instead. These glands were characterized by a layer of biliary epithelium surrounded by few connective tissue. Alongside with these findings, nests of hepatocyte cells with significant Alb expression were seen. Small interstitial fibrosis surrounding the glands was found in some areas. In glandular epithelial biliary cells, strong expression of K19 and Vim was observed, whereas it was decreased in undifferentiated...
glands. The expression of Alb was low in proliferating biliary cells. Vim positive fibroblasts and deposit of Col I and Col III were demonstrated in areas of fibrosis. The densitometric data showed higher expression of Alb and K19 in hepatocytes (p<0.05), compared with glandular epithelial biliary duct proliferations. Besides, significantly higher Vim, Col I and Col III expression was demonstrated in the epithelial-mesenchymal transition and in areas with hepatic fibrosis.

**Conclusions:** Experimental bile duct ligation induces a prominent biliary gland proliferation associated with hepatic fibrosis. Our data suggest that hyperplasia of intrahepatic biliary epithelial cells plays a key role at the beginning of the epithelial glandular transformation. However, the presence of Alb in glandular epithelial cells may be due to the potential of immature hepatocytes and undifferentiated mesenchymal cells present in fibrotic areas to transform into biliary cells. These molecular profiles of biliary gland proliferation in experimental cholestasis will allow future human cholestasis research.

**Disclosure of Interest:** None Declared
INFLUENCE OF THROMBOPHILIC GENE POLYMORPHISMS ON FIBROSIS PROGRESSION RATE IN CHRONIC HEPATITIS C

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Introduction: Nowadays, much attention is paid to the search for genetic factors which can explain the course of chronic hepatitis C (CHC). Hypercoagulation plays an important role in the progression of liver fibrosis, but the influence of coagulation encoding genes and pathways on the rate of the disease progression is poorly understood.

Aims: In this study we assess the prognostic and clinical value of the presence of different gene polymorphism combinations including FII 20210 G/A, FV 1691 G/A, FVII 10976 G/A, FXIII 103 G/T, ITGA2 807 C/T, ITGB3 1565 T/C, FBG -455 G/A, PAI -675 5G/4G, MTHFR 677 C/T on fibrosis progression in patients with chronic hepatitis C (CHC).

Material and Methods: 187 patients with CHC were categorized as «fast» (fibrosis rate progression ≥0.13 fibrosis units/yr, n=89) and «slow» (fibrosis rate progression < 0.13 fibrosis units/yr, n=88) progressors. Assessment of gene polymorphisms was carried out by PCR quantification.

Results: Genotype GA FV 1691G/A was more commonly observed in the group of «fast progressors» compared to the group of «slow progressors» (10.11% vs 1.14%, OR=9.787, p=0.011). A trend for the minor A allele FV 1691 G/A was more frequently observed in the group with progressive fibrosis than in the group with a slow fibrosis progression rate (1.7% vs 5.56%, p=0.139). Genotype GA FII 20210 G/A and genotypes with 4G allele (5G4G + 4G4G) and 4G allele PAI -675 5G/4G were also more common in «fast
progressors» compared with «slow progressors», the frequency of detection of this genotype had a trend to statistical significance (p=0.118, p=0.112 and p=0.117 respectively). No associations were found between polymorphisms in rhw other genes and fibrosis rate. An integrated model of coding “profibrogenic” genotypes (FV 1691 G/A, FII 20210 G/A, PAI-I -675 5G/4G) showed that rate of liver fibrosis progression (units fibrosis/year) increased with total score (p=0.039), indicating on combined effect of these genes.

Conclusions: We have shown that the presence of mutant genotypes FV 1691 G/A, FII 20210 G/A, PAI-I -675 5G/4G is a factor that are associated with rapid fibrosis progression in chronic hepatitis C patients.

Screen 2: YI-MP-I12

IMPLICATIONS OF INTERLEUKINS IN LIVER FIBROSIS AND SUSTAINED VIROLOGICAL RESPONSE IN PATIENTS WITH VIRAL HEPATITIS C

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Introduction: Hepatitis C virus (HCV) infection cause progressive liver injury and lead to fibrosis and cirrhosis. Single Nucleotide Polymorphisms (SNPs) in IL-28B and IL-10 were associated with sustained virological response and liver fibrosis in chronic hepatitis C patients.

Aims: Assessing the predictive value in liver fibrosis of interleukin 10, clinical and laboratory factors.

Material and Methods: Study included 176 hepatitis C patients admitted in Gastroenterology Unit of Emergency County Hospital Craiova, Romania, treated with peg-interferon and with ribavirin, for 48 weeks, and were followed for 24 weeks after the treatment. RNA HCV was determined by RT-PCR at 0, 12, 24, 48 and 72 weeks of treatment. Liver fibrosis was evaluated by elastography (Fibroscan\textsuperscript{®}) and serological tests (APRI score). We used Custom Taqman SNP Genotyping Assays to determine IL-28B SNP rs12979860 (in all patients) and IL-10R SNP -1087 (in 133 patients). We evaluated sustained virological response (SVR) and liver fibrosis in correlation with interleukins SNPs, in controls and HCV patients.

Results: Moderate liver fibrosis (F0-F2) was encountered in 48 patients (36.09\%) and advanced/severe fibrosis in 85 patients (63.91\%). For advanced/severe fibrosis we identified the following positive predictors: platelet count <150,000/mL (OR: 15.81; p = 0.0075), male gender (OR: 5.19, p=0.1622). Age under 50 was a protective factor for advanced/severe fibrosis (OR = 0.07, p = 0.0329). AST had higher values in patients with advanced/severe fibrosis (85.18 IU/L) than in those with mild/moderate fibrosis (64.31 IU/L) (p=0.0235). IL-28B SNP was did not predict liver fibrosis. The IL-10 SNP
predicted liver fibrosis, as 66.67% of patients with GG genotype presented severe fibrosis (OR: 4.732, p=0.2880), as compared with 73.02% for the GA genotype (OR = 26.84; p = 0.0482) and 51.02% for the AA genotype. The APRI score predicted successfully the advanced liver fibrosis, as it was 0.72 in mild to moderate fibrosis and 1.30 in severe fibrosis (effect size r=0.20, p=0.1562). The genotypes of IL-28B correlated with sustained virological response (SVR), but there were no significant differences of SVR rates between IL-10 SNP genotypes (p=0.3090). Patients with moderate liver fibrosis (F0-F2) had an increased chance of SVR (OR=2.03, p=0.0257).

**Conclusions:** The polymorphisms of IL-10R are involved in the evolution of liver fibrosis in patients with chronic hepatitis C. Fibroscan® and APRI score are reliable methods for evaluation of liver fibrosis.

**Disclosure of Interest:** None Declared
Screen 3: YI-MP-180

VIROLOGIC RESPONSE TO INTERFERON-FREE THERAPIES AMELIORATES HCV-INDUCED PORTAL HYPERTENSION

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Introduction: Since a decrease in hepatic venous pressure gradient (HVPG) translates into a clinically meaningful benefit, it is considered an acceptable surrogate endpoint for etiologic therapies.

Aims: We aimed to investigate the impact of sustained virologic response (SVR) to interferon (IFN)-free therapies on portal hypertension in patients with paired HVPG measurements.

Material and Methods: One hundred and four patients with portal hypertension (HVPG≥6mmHg) who underwent HVPG and liver stiffness measurement before IFN-free therapy (baseline [BL]) were retrospectively studied. One hundred patients achieved SVR. The effect of SVR on portal pressure was investigated in patients who underwent HVPG and liver stiffness measurement after antiviral therapy (follow-up [FU]; n=60). To demonstrate the generalizability of our results, the course of liver stiffness, platelet count, and liver function tests was compared to patients who also achieved SVR, but did not undergo FU HVPG measurement (n=40).

Results: SVR to IFN-free therapies significantly decreased HVPG across all BL HVPG strata: 6-9mmHg (BL:7.37±0.28 vs. FU:5.11±0.38mmHg; -2.26±0.42mmHg; *P*<0.001), 10-15mmHg (BL:12.2±0.4 vs. FU:8.91±0.62mmHg; -3.29±0.59mmHg; **P**<0.001), and >16mmHg (BL:19.4±0.73 vs. FU:17.1±1.21mmHg; -2.3±0.89mmHg; *P*=0.018). In the subgroup of patients with a BL HVPG of 6-9mmHg, HVPG normalized (<6mmHg) in 63% (12/19) of patients, while no patient progressed to ≥10mmHg. Among patients
with a BL HVP G≥10mmHg, a clinically relevant HVP G decrease of ≥10% was observed in 63% (26/41) and 24% (10/41) had a FU HVP G<10mmHg.

Patients with Child-Pugh B were less likely to have a HVP G decrease (HR: 0.103; 95%CI:0.02-0.514; P=0.006), when compared to Child-Pugh A patients. In the subgroup of patients with BL CSPH, the relative change in liver stiffness (per %; HR:0.972; 95%CI:0.945-0.999; P=0.044) was a predictor of a HVP G decrease ≥10%.

The area under the receiver operating characteristic curve for the diagnosis of FU CSPH by FU liver stiffness was 0.931 (95%CI:0.865-0.997).

Changes in liver stiffness, platelet count, and liver function tests were comparable between patients with and without FU HVP G measurement, which supports the generalizability of our results.

**Conclusions:** SVR to IFN-free therapies ameliorates portal hypertension across all BL HVP G strata. However, amelioration of portal hypertension was less likely in patients with more advanced liver dysfunction. Transient elastography might be useful for the non-invasive evaluation of portal hypertension after SVR.

**Figure:**

![Image of graph showing changes in HVP G](image)

**Disclosure of Interest:** None Declared
NK CELLS FROM HOMOZYGOUS NLG4-/- (KO) MICE INHIBIT LIVER FIBROSIS THROUGH PI3K/AKT/MTOR PATHWAY AND DECREASED INTERLEUKIN-4

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Aims: We assessed the role of NLG4 in NK cell killing of liver fibrosis outcome.

Material and Methods: Naïve Wild-Type (WT) and homozygote NLG4-/- mice induced for CCl₄-fibrosis were evaluated for fibrotic and inflammatory profile.

Results: ALT serum levels were significantly lower in the NLG4-/- fibrotic mice. FACS analysis of liver lymphocytes showed increased NK cell count associated with increased lysosomal-associated-membrane-protein-1 (CD107a, NK activation marker) and a decrease in CD8 cells. Western blot analysis of aSMA quantitations showed resistance of NLG4-/- mice to fibrosis in both the acute and the chronic exposure to CCl₄ injections. Isolated pHSCs from livers of NLG4-/- mice showed decreased aSMA associated with their increased apoptosis (Annexin-V) and inability to proliferate (CFSE). In co-culture conditions, NK with NLG4-/- receptor showed increased adherence/phagocytosis to WT pHSCs as well as increased expressions of the PI3K/AKT/mTOR mRNA (p=0.002). These data was associated with decreased IL-4. NK with NLG4-/- receptor transplanted in CCl₄-induced irradiated mice inhibited progressions of liver fibrosis as compared of NK from naïve mice.

Conclusions: Homozygous NLG4-/- mice exert anti-fibrotic profile through increase in NK cells and decreased CD8 cells. Alterations in NK cells phenotypes were observed through increased activity associated with increased pHSCs killings and consequently decreased in aSMA quantitations. NK activations and potentials to kill are suggested to be associated with elevated expressions of PI3K/AKT/mTOR accompanied in decreased in the pro-fibrogenic marker; IL4. NLG4 modulations could be a potential target for fibrosis treatments.

Disclosure of Interest: None Declared
HEPATIC STELLATE CELLS ARE THE MAIN SOURCE OF MYOFIBROBLASTS IN A MOUSE MODEL OF CHRONIC CHOLESTATIC LIVER DISEASE

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Introduction: The cellular source of collagen in chronic liver disease has been discussed controversially in the recent past. Hepatic stellate cells are the major source of collagen in toxic liver injury. Portal myofibroblasts, bone marrow and epithelial cells (hepatocytes and cholangiocytes) have been suggested as alternative sources of myofibroblasts in chronic liver disease. Mice with genetic disruption of the ATP-binding cassette transporter B4 (abcb4) lack phosphatidylcholine excretion into bile associated with accumulation of toxic bile acids. Abcb4 KO mice mimic sclerosing cholangitis in humans and represent a valuable model to investigate the progression of cholestatic liver disease and the development of liver cancer in a fibrotic and inflammatory environment.

Aims: The aim of this study was to characterize collagen-producing myofibroblasts in a mouse model of sclerosing cholangitis.

Material and Methods: Coll-GFP reporter mice in which the collagen α1(I) promoter drives expression of a green fluorescent protein (GFP) were crossed to abcb4 KO mice. The mice were analyzed for expression of cell type specific markers by immunofluorescence and confocal microscopy. GFP+ cells were sorted from livers of Coll-GFP/abcb4 KO mice at different time points and gene expression was analyzed by microarray technology.

Results: GFP+ cells lacked expression of epithelial cell markers keratin 8 and 19 and the endothelial cell marker CD31. In contrast, 80.3% of collagen producing cells stained positive for desmin, a marker typical for hepatic stellate cells.

Conclusions: Hepatic stellate cells are the major source of collagen producing cells in cholestatic abcb4 KO mice.

Disclosure of Interest: None Declared
COMPARATIVE STUDY OF HEPATIC FIBROSIS REGRESSION IN INTERFERON BASED VERSUS INTERFERON FREE REGIMENS RESPONDERS IN HEPATITIS C THERAPY USING FIBROSCAN DEVICE

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Introduction: Hepatitis C virus (HCV) infection usually progress to chronic infection with subsequent cirrhosis and cancer. Therapies aim to eradicate the virus and prevent further progression. Interferon is claimed to have anti-fibrotic effect.

Aims: This prospective study aimed to assess the effect of interferon based versus interferon free antiviral regimens on fibrosis stages in HCV patients using Transient elastogram (Fibroscan).

Material and Methods: Prospective observational cohort study included two groups group 1: 150 chronic HCV patients on interferon based regimen group and group 2: 150 Chronic HCV patients on interferon free regimen group. They were examined using fibroscan (Echosens, Paris, France, device 502, M probe) before, 12, 24, 36 and 48 weeks of therapy. The results of fibroscan were compared to each other and with other group.

Results: The fibrosis results had regressed significantly after 36 weeks of starting therapy of interferon based regimens patients’ groups I (p<0.05) with more regressions after 48 weeks. When categorized by response to therapy, Responders showed significant decline in their fibroscan scores compared to non-responders of same fibrosis degree. While in group II, the significant regression in fibrosis stages started after 48 weeks and with significant difference than interferon based regimens group.

Conclusions: The degree of fibrosis regresses significantly in HCV responders on anti-viral INF based regimens earlier and with significant different than INF free regimens in treatment chronic HCV.

Disclosure of Interest: D. Ziada: Grant: A project funded by Science and Technology Development Fund, Egypt, Grant No.15183. , H. SOLIMAN: Grant: A project funded by Science and Technology Development Fund, Egypt, Grant No.15183. , M. anees: None Declared
HEPATIC STELLATE CELLS ARE THE MAJOR SOURCE OF COLLAGEN IN MURINE MODELS OF LIVER FIBROSIS

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Introduction: Hepatic stellate cells (HSCs) are the major storage site for vitamin A in vertebrates and are the major source of collagen in toxic liver injury. Lecithin-retinol acyltransferase (LRAT) catalyzes the esterification of all-trans-retinol and is essential for storage of vitamin A in the liver. Other sources of myofibroblasts have been proposed in fibrotic livers including bone marrow derived cells and epithelial cells (hepatocytes and cholangiocytes). Portal fibroblasts appear to be of particular importance in cholestatic liver disease.

Aims: This study addressed if Lrat is a specific marker for HSCs and evaluated the contribution of HSCs to the pool of collagen-producing cells in different mouse models of liver fibrosis.

Material and Methods: BAC transgenic Lrat-Cre mice were generated by insertion of a codon optimized Cre recombinase at the translational start of the lrat gene by ET cloning. Lrat-Cre mice were crossed to reporter mice, which express tdTomato after removal of a loxP-flanked STOP cassette. Triple transgenic mice (Coll-GFP/Lrat-Cre/ROSA26 LSL tdTomato) were generated and subjected to BDL or CCl4 treatment. Immunofluorescence staining and confocal microscopy was employed to assess the relative contribution of individual cell types to the pool of collagen-producing cells.

Results: Immunofluorescence staining for desmin indicated that Lrat-Cre marks >95% of HSCs. Co-localization analysis of triple transgenic mice indicated that >90% of collagen-producing cells in mice subjected to CCl4 treatment or BDL are derived from HSCs. Neither collagen-producing cells nor HSC (desmin-positive cells/ tdTomato-positive cells
in Lrat-Cre ROSA26 LSL tdTomato) expressed markers of hepatocytes, cholangiocytes or endothelial cells.

**Conclusions:** Our study suggests that 1) Lrat is a specific marker for HSCs, 2) Lrat-Cre mice are a valuable tool to study gene function in HSCs and 3) HSCs are the main source of collagen in mice undergoing BDL and CCl4 treatment.

**Disclosure of Interest:** None Declared
TRANSCRIPTOME PROFILING OF HSCS FRESHLY ISOLATED FROM YOUNG AND OLD DONORS SUGGESTS AN ENRICHMENT OF INACTIVATED HSCS WITH AGE

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Introduction: A well-established risk factor for the incidence and progression of liver fibrosis is advanced age. However, to date the cellular and molecular mechanisms that underlie age-related susceptibility to liver fibrosis remain poorly understood. The resident hepatic stellate cell (HSC) has unambiguously been identified as the predominant source of myofibroblasts in the fibrotic liver, irrespective of the underlying disease etiology.

Aims: In the present study, we compared freshly isolated HSC populations from young and old livers with the aim to identify qualitative differences that might explain the higher incidence and progression rate of liver fibrosis with age.

Material and Methods: Freshly isolated, non-cultured human primary HSCs from young (≤12 years; n=3) and old donors (≥51 years; n=4) were compared by gene expression profiling using Affymetrix HG-U219 genechips. HSCs in liver tissue from young and old donors were analyzed by immunohistochemistry using vimentin.

Results: We find that despite a same level of quiescence, over 1700 genes are differentially expressed between young and old HSCs (P<0.01; FC≥2). Half of these genes are enriched in old HSCs and pertain to response to wounding, regulation of cell activation and includes Cathepsin S (CTSS), a lysosomal cysteine proteinase previously identified as a signature gene of inactivated HSCs (iaHSCs). We confirm the increase of CTSS+ HSCs by immunohistochemistry in mice recovered from CCl₄-induced fibrosis and find that CTSS expression inversely correlates with the expression of activation markers in human
primary HSCs. In human livers, CTSS is almost exclusively expressed in HSCs and not in portal fibroblasts. Quantitatively, CTSS expression is more abundant in the livers of old patients and found to be increased in perisinusoidal HSCs from young patients with early-stage fibrosis. This differential expression is not linked to a difference in activation status, as CTSS expression is not detected in activated myofibroblasts in patients with drug-induced, alcohol-induced and HBV-induced liver cirrhosis.

**Conclusions:** Our study for the first time identifies CTSS as a potential marker of human iaHSCs and finds that CTSS+ HSCs are enriched in livers of older patients. In light of previous studies showing that iaHSCs are primed to differentiate into myofibroblasts, an accumulation of iaHSCs might contribute to the accelerating rate of fibrosis with advancing age.

**Figure:**

![Image of histological sections showing CTSS expression in old and young patients](image)

**Disclosure of Interest:** None Declared
THE ROLE OF CIRCULATING MICROVESICLES IN PROGRESSIVE LIVER INJURY

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Introduction: Circulating microvesicles (cMVs) are extracellular nanovesicles secreted from cell membrane and released by numerous cell types. They are enriched with nucleic acids including messenger RNA (mRNA) and microRNA (miRNA). Multiple miRNAs are shown to be involved in several regulatory steps leading from inflammation to fibrosis and hepatocellular carcinoma.

Aims: We aim to investigate the utility of cMVs as plasma biomarker for liver disease condition such as inflammation and fibrosis.

Material and Methods: Microvesicles were isolated from human plasma using differential centrifugation and enumerated by NanoSight. Total RNA form cMVs was extracted from patient with non-diseased liver F0 (n=5), hepatitis C virus-related fibrosis F1-F2 (n=8) and liver cirrhosis F4 (n= 13). miRNA expression was measured with the nCounter system (NanoString). Differentially expressed miRNAs were selected, and target genes and their signalling pathways were predicted with Ingenuity software.

Results: Several cMVs miRNAs were deregulated in the plasma compared to healthy controls and correlated with the degree of liver fibrosis. Four miRNA expression level differed among liver fibrotic stages (p<0.05). The miRNA target genes were involved in deregulation of Calcium Signaling, Protein Kinase A Signaling and ERK5 signaling pathways.

Conclusions: Plasma extracted cMVs might reveal the new insight into liver disease progression and be used as a molecular marker for monitoring liver fibrosis and severity of cirrhosis.

Disclosure of Interest: None Declared
IMPACT OF FIBROSIS STAGE AND OTHER RISK FACTORS FOR DEVELOPMENT OF HEPATOCELLULAR CARCINOMA (HCC) AFTER SUCCESSFUL TREATMENT OF HEPATITIS C WITH INTERFERON α.

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Introduction: There is a debate on whether surveillance for HCC (Hepatocellular Carcinoma) should be performed in patients with liver fibrosis following a SVR (Sustained Virological Response) for hepatitis C.

Aims: The aim of the present study was to investigate a possible correlation between HCC development and the liver fibrosis pre-treatment stage, age, alcohol consumption, and some laboratory parameters, in patients, successfully treated for chronic HCV infection.

Material and Methods: The records of 357 (284M, 73F) consecutive patients that underwent successful treatment for chronic HCV infection with pegylated interferon α and ribavirin between the years 2004-2014 were reviewed. Age, sex, alcohol consumption, viral genotype and pre-treatment levels of viral load, platelets, bilirubin, γ-globulins, INR, HbA1c, AFP, CA 19-9 and also fibrosis stage were taken into account in the performed multivariate analysis. Patients were followed routinely every six months with liver ultrasound and AFP levels. The median follow up period after SVR was 62 months (range 11-120).

Results: During the follow-up period 14(11M, 3F, 3.92%) out of 357 patients developed HCC. The median period from SVR to HCC diagnosis was 35 months (range 21-139). Alcohol consumption (HR 7.3: 95% CI 3-15.9; P<0.0001), age ≥55 years (HR 3.3: 95% CI 1.5-6.4; P=0.009) and advanced (F3/F4) fibrosis stage (HR 3.7: 95% CI 1.8-7.6; P<0.0001) were identified as independent and statistically significant factors for development of HCC after successful eradication of HCV.
Conclusions: Pretreatment advanced fibrosis stage, alcohol consumption and age>55 years can consider as significantly important factors correlated with the development of HCC in non-viremic HCV patients prior treated with interferon a and ribavirin.

Disclosure of Interest: None Declared
Screen 1: YI-MP-195

25(OH) D3 ALLEVIATE LIVER NK CYTOTOXICITY IN EARLY BUT NOT IN LATE FIBROSIS MODEL OF BALB/C MICE DUE TO MODULATIONS IN VITAMIN D RECEPTOR

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Introduction: CD8-subsets mediate hepatic-fibrosis and NK-cells exert anti-fibrotic effects via direct interactions with hepatic-stellate-cells (HSC); mediated by phagocytosis. Low 25-Hydroxy-vitamin-D; “25(OH) D3” serum and vitamin D receptor (VDR) levels were recently correlated to advanced fibrosis in Chronic Hepatitis C (CHC). However, vitamin D receptor (VDR) mechanism in liver fibrosis modulations is not well understood.

Aims: We aimed to evaluate associations of VDR with NK cell modulations during fibrogenesis.

Material and Methods: Carbon-tetrachloride (CCl₄) hepatic-fibrosis was induced in Balb/c -mice for 4 weeks. Along 1th to 4th weeks; 25 (OH) D3 or sham solutions were i.p injected/2x week. Liver NK cells were isolated and were identified as resident (CD49a+/CD49b-) NK1.1. NK cells were further stained for CD107a (LAMP-1; Lysosomal-associated membrane protein 1) as a marker for activation and analyzed by flow-cytometry. Liver proteins were quantified for VDR and aSMA expressions by western blot and RT PCR, respectively. Livers were also assessed histologically and serum ALT levels were estimated.

Results: Hepatic fibrosis were gradually increased along 4 weeks injections of CCl₄ as indicated by serum ALT levels and aSMA expressions (P<0.02) as well as H&E staining of liver necro-inflammatory lesions. These results were associated with increased activations of NK1.1 CD107a. While 25(OH) D3 administrations did not modulate fibrosis of early stages (weeks 2 & 3); 25(OH) D3 significantly worsen hepatic-fibrosis of late stage (week 4) as hepatic aSMA expressions and serum ALT levels increased. In week 4, no further
activations of NK cells were seen following 25(OH)D3 injections and were associated with down expressions of VDR (Fold-1.7, P<0.01) indicating the inability of 25(OH)D3 to ameliorate hepatic fibrosis were lost due to modulations in VDR.

**Conclusions:** 25(OH) D3 alleviate liver NK cytotoxicity in early but not in late fibrosis model of Balb/c mice due to modulations in vitamin D receptor. More studies are needed to clarify the role of VDR in attenuation of liver fibrosis.

**Disclosure of Interest:** None Declared
INHIBITION OF MIRNA-21 PREVENTS DEVELOPMENT OF FIBROSIS AND AMELIORATES NALFD PATHOGENESIS IN MICE

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Introduction: Fibrosis represents one of the most critical events during non-alcoholic fatty liver disease (NAFLD) pathogenesis and microRNAs (miRNA/miRs) were recently proposed as novel pathogenic factors in the development of fibrosis and NAFLD progression.

Aims: We aimed to elucidate the role of miR-21, and the modulation of its target peroxisome proliferator-activated receptor alpha (PPARα), during NAFLD pathogenesis in mice.

Material and Methods: C57BL/6 wild-type (WT) and miR-21 knockout (KO) mice were fed with a chow diet (n=10) or a methionine and choline-deficient diet (MCD; n=10) for 2 and 8 weeks. Human liver biopsies were obtained from morbid obese NAFLD patients at different disease stages (n=28). Liver samples were processed for histology and analysis of miR-21, pro-inflammatory/pro-fibrogenic cytokines and PPARα, by qRT-PCR and immunoblotting.

Results: WT mice fed the MCD diet developed steatosis and displayed increased levels of TNF-α, IL-1β and IL-8 along with increased serum ALT and AST levels, consubstantiating NASH development. Importantly, miR-21 expression levels were significantly increased, concomitantly with decreased PPARα levels, a correlation also found in NAFLD patients, further increasing from steatosis to less and more severe NASH. Also, particularly at 8 weeks, WT MCD-fed mice developed fibrosis and presented increased levels of pro-fibrogenic cytokines TGF-β, collagen 1α1 and αSMA. In contrast, miR-21 KO mice...
displayed a significant decrease in steatosis severity, liver damage and inflammation, along with increased levels of PPARα. Importantly, miR-21 KO MCD-fed mice did not develop fibrosis, concomitantly with a complete reversion on the levels of pro-fibrogenic cytokines to control levels.

**Conclusions:** In conclusion, our results indicate that miR-21 downregulation, likely leading to increased PPARα, ameliorates NASH pathogenesis in mice, decreasing steatosis, inflammation and preventing the development of fibrosis, thus highlighting its therapeutic potential. (Supported by PTDC/BIM-MEC/0873/2012, SFRH/BD/88212/2012 and SFRH/BD/91119/2012, FCT, Portugal).

**Disclosure of Interest:** None Declared
JUNCTIONAL ADHESION MOLECULE-A DEFICIENCY LEADS TO PROFIBROTIC EFFECTS IN TWO EXPERIMENTAL LIVER FIBROSIS MODELS IN MICE

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Introduction: Liver fibrosis is associated with infiltrating immune cells and activation of hepatic stellate cells leading to an excess production of extracellular matrix proteins. Junctional Adhesion Molecule-A (JAM)-A is an adhesion molecule, expressed on endothelial, epithelial cells (tight junctions) and also on leukocytes, especially on T cells, monocytes and neutrophils (recruitment and trafficking).

Aims: Here we aimed to investigate the effects of JAM-A deficiency during the development of liver fibrosis in two different fibrosis models.

Material and Methods: Liver fibrosis was induced in JAM-A-/- and wild type (WT) mice by repetitive intraperitoneal injections of carbon tetrachloride (CCL₄) over 6 weeks and by bile duct ligation (BDL, 2 weeks). Mice were sacrificed and liver fibrosis was analysed by histology (Sirius Red staining), intrahepatic expression of fibrosis-related genes (TGF-β, Col1α1, MMP-9, TIMP-1, α-SMA) and fibrosis-related proteins (Collagen I and α-SMA). To quantify the infiltration of immune cells we performed flow cytometry. Moreover, we analyzed the immune response via TNF-α and IL-1β qRT-PCR.

Results: In both fibrosis models, JAM-A deficient mice were more prone to liver fibrosis when compared to WT mice (Fig. 1), as assessed by analysis of Sirius Red stained liver sections. Also, activation of hepatic stellate cells was increased in our models. We could validate these results on mRNA- and protein-level. Moreover, flow cytometry analysis demonstrated a higher number of infiltrating monocyte-derived macrophages in JAM-A-/- mice compared to WT mice (P < 0.05). The mRNA levels of the pro-inflammatory cytokines TNF-α and IL-1β were also increased in JAM-A-/- mice.
**Conclusions:** Our results showed that \( \text{JAM-A} \) deficiency led to a deterioration of liver fibrosis induced by higher hepatic stellate activation and increased monocyte-derived-macrophage infiltration into the liver. Thus, therapeutic modulation of JAM-A might be a promising target for chronic liver diseases.

**Figure:**

![Image showing Sirius Red staining of JAM-A\(^{-}\) and WT liver tissue after treatment with CCl\(_4\) or BDL](image)

Liver fibrosis was induced in wild type (WT) and Junctional Adhesion Molecule-A (JAM-A)\(^{-}\) mice by intraperitoneal injections of carbon tetrachloride (CCl\(_4\)) over 6 weeks or by bile duct ligation (BDL). Via Sirius Red staining the level of fibrosis was determined in WT, JAM-A\(^{-}\) and control (ctrl) mice.

**Disclosure of Interest:** None Declared
CHRONIC ADMINISTRATION OF THE NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS Efavirenz and Rilpivirine Decreases Hepatic Steatosis and Fibrogenic Response in a Mouse Model of NAFLD

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Introduction: Non-alcoholic Fatty Liver Disease (NAFLD) is one of the most frequent causes of hepatic disease, and its incidence is higher in HIV patients due to the viral infection and chronic antiretroviral therapy. The role of these drugs in NAFLD has not yet been clarified, but some of them have been associated with hepatotoxicity. Efavirenz (EFV), one of the most widely used antiretrovirals, induces mitochondrial dysfunction, endoplasmic reticulum stress and metabolic and inflammatory changes in hepatocytes.

Aims: To characterize the effects of the non-nucleoside reverse transcriptase inhibitors EFV and rilpivirine (RPV) in the progression of NAFLD in a mice model.

Material and Methods: C57BL/6 mice were fed a normal diet (ND) or high fat diet (HFD) as a model of NAFLD (12 weeks). EFV and RPV were administered daily (p.o.) to the mice using dosages equivalent to those in HIV patients, and a weekly control was carried out. Serum levels of lipid metabolic parameters were measured, and hepatic expression of specific markers of inflammation (Caspase-1, NLRP3, IL1β, IxBα), lipid accumulation (ADFP, PPARγ) and fibrogenesis (Col-1, αSMA, TIMP-1, TGFβ, MMP2 and Vimentin) were analysed by qPCR and Western Blot. Different histological analyses were performed to determine lipid infiltration (haematoxylin and eosin) and fibrogenesis (sirius red).

Results: HFD-fed mice showed an increase in their total and hepatic weight in comparison with ND-fed mice. EFV decreased total weight in both groups. With respect to lipid metabolism, this compound increased total serum cholesterol levels and reduced liver lipid content and hepatic expression of ADFP and PPARγ. EFV also significantly decreased expression of fibrogenic genes and collagen deposition in both ND and HFD.
groups. Moreover, the drug diminished the expression of proinflammatory markers (Caspase-1 and NLRP3) only in the ND groups, an effect that was not observed in the HFD groups. RPV-induced effects in inflammatory and fibrogenic responses were similar to those exerted by EFV.

**Conclusions:** EFV and RPV did not aggravate the progression of NAFLD in this *in vivo* model; in fact, EFV had an unexpected antifibrogenic, antiinflammatory, and antiadipogenic effect in the liver. Although further studies are needed to characterize the specific effects of these drugs in different hepatic cellular populations, these preliminary results may help to select the most appropriate combinations of antiretrovirals in HIV-infected patients with particular susceptibility to liver diseases.

**Disclosure of Interest:** None Declared
PTX3 IS A NOVEL MARKER OF DISEASE PROGRESSION IN ALCOHOLIC HEPATITIS AND ATTENUATES LPS-INDUCED LIVER INJURY AND INFLAMMATION

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Introduction: Pentraxin-3 (PTX3) is a component of the innate immunity and an important regulator of the immune system. However, the role of PTX3 in the liver and its potential participation in tissue injury is still unknown. Alcoholic hepatitis (AH) is characterized by an acute and severe liver inflammation and may progress to an acute-on-chronic liver failure.

Aims: The aim of this study was to investigate the potential of PTX3 as a biomarker in AH and its role in wound-healing response.

Material and Methods: PTX3 plasma levels (n=56) and liver gene expression (n=30) were assessed in patients with AH and in experimental models. PTX3 expression was down-regulated in vitro by interference RNA. Effects of recombinant PTX3 were evaluated in cells cultures, in ex-vivo tissue slices and in a mouse model of acute-on-chronic liver damage (chronic CCl4 and LPS). Gene expression of inflammatory mediators was assessed by quantitative PCR, and inflammatory cell recruitment was determined by immunohistochemistry.

Results: PTX3 hepatic gene expression and plasma levels were increased in patients with severe AH compared to mild AH, cirrhotics and healthy patients (p<0.01). PTX3 correlated with clinical scores of severity (MELD, ABIC), endotoxaemia and short-term mortality (p<0.05). Moreover, PTX3 expression was up-regulated in experimental models of chronic CCl4 treatment and acute-on-chronic liver injury. Cultured activated HSCs and macrophages expressed PTX3 that was up-regulated upon stimulation with pro-inflammatory agents (TNFα, IL1β, LPS). While knock-down of PTX3 in HSCs reduced activation, stimulation with recombinant PTX3 induced ERK and AKT-phosphorylation.
and expression of activation markers. Next, we evaluated the effect of PTX3 on fibrotic liver tissue slices showing that PTX3 attenuated LPS-induced liver injury and expression of inflammatory mediators. Moreover, recombinant PTX3 administration reduced LPS-induced liver injury, inflammatory response and inflammatory cell recruitment in a mouse model of acute-on-chronic liver injury.

**Conclusions:** We show that PTX3 is a novel marker of disease progression in AH strongly associated with LPS serum levels. Activated HSCs produce and respond to PTX3 promoting its activation. Ex-vivo and experimental mouse models suggest that PTX3 attenuates liver injury and inflammatory response to LPS. These results suggest that PTX3 is a new marker of disease progression in AH and has a protective role in acute-on-chronic liver injury.

**Disclosure of Interest:** None Declared
NOVEL LOW-COST AND EFFICIENT METHOD FOR THE ISOLATION OF MOUSE LIVER SINUSOIDAL CELLS

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Introduction: Liver disease encompasses more than just the damage of one, or activation of another cell type to cause an imbalance of liver homeostasis. Upon hepatocyte injury, Kupffer cells (KCs), hepatic stellate cells (HSCs) and sinusoidal endothelial cells (LSECs) try to collectively cope with the altered situation. Complex culture systems can mimic this in vitro and several groups developed methods for the isolation of sinusoidal cells from mice using specific antibodies and fluorescence activated cell sorting (FACS). The disadvantage of these techniques are that they are time consuming and expensive.

Aims: We aimed to develop an isolation procedure of liver sinusoidal cells based on their scavenging features.

Material and Methods: Before liver cell isolation, male Balb/c mice were i.v. injected with secondary antibodies, coupled to alexa 647 (APC) fluorochromes. Livers were enzymatically digested to single cell suspensions and from the non-parenchymal fraction, KCs and LSECs were isolated using a FACS Aria II, on the basis of their UV negativity and APC positivity. HSCs were sorted on the basis of the retinyl ester autofluorescence at 328 nm (UV+). The method was also used with mice that underwent a bile duct ligation (10 days) or were treated with carbon tetrachloride (4 weeks).

Results: After injecting mice with fluorescent antibodies, LSECs (28.7%) and KCs (12.7%) populations could clearly be distinguished on APC-UV FACS-plots. These populations were isolated and their purity was verified using mRNA levels and by immunohistochemical analysis of liver cell specific markers of the sorted cells. Ideal dose and injection time point were determined at 10 μg of fluorochrome coupled antibodies 2 hours before isolation. Although slightly less pure, such isolation can also be used for fibrotic livers. In addition, we observed that 15.1% ± 2.7% of the LSECs are positive for UV autofluorescence, normally used to detect HSC by FACS. Excluding these UV+
APC+ cells from the total UV fraction, allowed us to obtain ultra-pure HSCs.

**Conclusions:** We established an easy, rapid and cost efficient method to isolate highly pure KCs, LSECs and HSCs from mouse livers. The procedure, including perfusion, takes only 90 minutes to obtain the different cell types. On average, the costs are at least 50 euros less per mouse when compared to conventional primary antibody-based methods. This method will make in vitro studies of sinusoidal cells more accessible to the entire research community.

**Figure:**

![Figure](image)

**Disclosure of Interest:** None Declared
RECOMBINANT \( \beta \)-NEUROXIN AS A THERAPEUTIC TARGET TO INHIBIT FIBROSIS THROUGH ALLEVIATION IN NK CELLS CYTOTOXICITY FOLLOWING INHIBITION OF NEUROLIGIN-4 (NLG4) RECEPTOR

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Introduction: Neuroligin-4 receptors (NLG4) are postsynaptic adhesion proteins that control the maturation and function of synapses in the central nervous system (CNS). NK cells from Nonalcoholic Fatty Liver Disease (NAFLD) patients exert high expressions of NLG4 and are thought to interfere with NK cell activity. NLG4 receptor interacts through immune synapse with its ligand \( \beta \)-neuroxin (cell-adhesion molecules) and is thought to play an important step in fibrogenesis.

Aims: We investigated a potential role of recombinant \( \beta \)-neuroxin to inhibit NLG4 elevations of NK cells in an in vitro co-culture condition.

Material and Methods: NK cells isolated from peripheral blood of NAFLD patients lacking metabolic syndrome were pre-incubated with 4 and 10 nM recombinant \( \beta \)-neuroxin for 3 hours prior to incubations with hepatic stellate cells (HSCs; LX2-cell line). Following 24 hours, cells were trypsinized and analyzed by flow-cytometry for NK activity by CD107a (Degranulation marker- a marker for NK activation) and LX2 activities (\( \alpha \)-smooth-muscle intensities). Interlukine-4 (IL4) expressions were also assessed in NK cells.

Results: LX2 cell line express 75% of \( \beta \)-neuroxin. In co-cultures, recombinant \( \beta \)-neuroxin significantly decreased the receptor expressions of NLG4 on NK cells and was well correlated with the increase in the recombinant concentrations. In additions, NK cells showed significantly increased lysosomal-associated membrane protein-1 (CD107a,
NK activation marker) from 13% to 50% and 53% with the 4 and 10 nM recombinant, respectively (P<0.03). Compared to LX2 mono-cultures, elevations of NK cells CD107a activity was associated with increased LX2 killing; as αSMA mean fluorescence intensities of HSCs decreased from 2948 in cultures without the recombinant to 2552 and 2066 in cultures with the 4 and 10 nM recombinant, respectively, (P<0.01). NK cells pre-treated with the recombinant b-neuroxin showed decreased in IL-4 secretion in both the concentrations (2-folds, P=0.04).

**Conclusions:** β-neurexin-NLG4 recognition mediates HSCs-NK immune synapse to control release of NK vesicles. Recombinant b-neuroxin activates NK cells to promote anti-fibrotic effects through increased HSCs killing. These effects were associated with decreased expression in the NLG4 receptor as well as with inhibition of the pro-fibrogenic marker IL4 in the NAFLD NK cells. NLG4 modulation of CD107a activity of NK cells extends the understandment and therapeutic strategies in fatty liver disease.

**Disclosure of Interest:** None Declared
Screen 2: YI-MP-I97

TGF-BETA2 INHIBITION IN ENDOTHELIAL CELLS AMELIORATES LIVER FIBROGENESIS AND INFLAMMATION

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Introduction: Development of new therapies for CLD is still an open issue given high mortality of HCC & organ-donor limitation. TGFb1 is a key cytokine often overexpressed in CLD including all stages from onset of liver injury, inflammation & fibrosis to end stage cirrhosis & HCC. AntiTGFb strategies basing on TGFb1 inhibition were not progressed further in clinical trials for liver fibrosis.

Aims: After providing evidence that TGFb2 is deregulated in mouse & human fibrogenesis we aimed to selectively target TGFb2 expression using antisense oligonucleotides (AONs) for attenuation or blockage of liver disease progression.

Material and Methods: 2 mouse models were investigated representing different types of CLD background. TGFb2 was specifically targeted using 17mer AON. Chronic liver damage was induced i.p. with CCl4 injections twice/week for 4 weeks. After 2 weeks subcutaneous AON application started in parallel twice per week. In MDR2KO mice AON was administered for 4 weeks. The effect & efficacy of AON treatment was evaluated by liver tissue morphology, on protein & mRNA level & plasma. Fibrotic and inflammatory markers were investigated. AON biodistribution was examined by co-IF stainings of specific cell type markers and labelled AONs.

Results: In vivo AON distribution revealed strongest signals in liver & kidney after 24h & 5d. Localisation of AON in the liver was found to be predominantly in LSECs & to less extend in Kupfer & stellate cells but not in hepatocytes. Simultaneous isolation of 4 liver cell types revealed LSECs, stellate and Kupfer cells as source of TGFb2. AON treatment
showed no toxic effects as assessed by liver plasma parameters & body weight. CCl₄ injured & MDR2KO AON-treated animals showed reduced TGFb2 mRNA in liver. Significant changes on fibrogenic & inflammatory processes were detected. Hydroxyproline was significantly reduced in both injury models. Sirius Red staining uncovered significant reduction (~34-54%) of collagen deposition. QPCR analysis of MDR2-KO livers showed reduction of TNFα, IL6 & PPARy expression & inflammatory infiltrates. Upon AON treatment only little impact was observed on aSMA and TGFb1 levels. In CCl₄ model significant reduction of stellate cell activation (~50%) was seen upon AON treatment as assessed by αSMA protein expression.

**Conclusions:** In vivo application of TGFb2 directed AONs to CLD mouse models contributed to attenuation of fibrogenesis. Further studies are currently performed to determine mechanistic details of AON effects and define specifications of a potential AON based treatment of CLD.

ASSOCIATION OF CONTROLLED ATTENUATION PARAMETER AND GLYCOSYLATED HEMOGLOBIN IN PATIENTS WITH FATTY LIVER

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Introduction: Chronic liver diseases and serum glycosylated hemoglobin (HbA1c) levels are linked to one another through the metabolic syndrome.

Aims: Our aim now was to analyze for the first time the potential association between hepatic steatosis, as determined quantitatively with the controlled attenuation parameter (CAP), and HbA1c.

Material and Methods: At a tertiary referral center in Germany, we evaluated a group of 212 outpatients with hepatic steatosis retrospectively, of whom 93.4% presented with non-alcoholic liver disease. Hepatic steatosis was assessed non-invasively using controlled attenuation parameter (CAP), which quantifies the degree of ultrasound attenuation based on vibration-controlled transient elastography (VCTE, Fibroscan). Serum HbA1c and liver function tests were measured with standardized clinical chemistry assays. The prosteatotic gene variant \textit{PNPLA3} p.I148M was genotyped using \textit{Taqman} assays.

Results: Overall in this cohort (113 men, median age 52 years), median CAP was 293 dB/m (100–400), and 171 (80.7%) patients presented with elevated CAP ≥ 238 dB/m, indicating marked hepatic steatosis. Median BMI was 30.2 kg/m² (17.2–47.4), median HbA1c was 5.6% (3.7–10.4) and serum ALT activities were 45 U/l (9–301). The frequency of elevated CAP increased with higher serum HbA1c levels ($r_s = 0.230$, $P = 0.001$). Patients with both hepatic steatosis and increased HbA1c levels (HbA1c ≥ 6.0%) displayed significantly ($P = 0.001$) higher CAP values as compared to those with normal levels (312 vs. 286 dB/m). In our cohort, 104 patients (49.0%) carried at least one \textit{PNPLA3} p.148M risk allele. When stratifying for the patient’s \textit{PNPLA3} genotype, the genetic association was maintained for carriers of the risk allele p.148M and normal levels of HbA1c ($P < 0.001$) but not for those with increased levels. Overall, the risk for hepatic
steatosis was independently associated with HbA1c, BMI, ALT and age as determined by multivariate linear regression analysis (all \( P \leq 0.013 \)).

**Conclusions:** Non-invasive risk stratification and follow-up of fatty liver in patients with metabolic syndrome is needed because of potential progression to steatohepatitis. Steatosis as assessed by CAP is associated with HbA1c in non-diabetic individuals, and the combination of these non-invasive markers improves individual risk assessment of patients with chronic liver diseases.

**Figure:**

![Scatter Plot](image)

**Disclosure of Interest:** None Declared
EARLY CHANGES IN NON-INVASIVE ASSESSMENT OF LIVER FIBROSIS IN HEPATITIS C VIRUS-INFECTED PATIENTS TREATED WITH DAAS: PRELIMINARY REPORTS

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Introduction: Chronic hepatitis C is a major cause of liver-associated mortality caused by decompensated cirrhosis and HCC. With the approval of DAA regimens, therapeutic efficacy has markedly increased. Early changes in non-invasive assessment of liver fibrosis under effective antiviral therapy are widely unknown and literature is still poor.

Aims: Our aim was to evaluate early changes of fibrosis determined by liver stiffness measurement (LSM) in patients treated with different DAA regimens.

Material and Methods: A total of 68 HCV pts were treated prospectively with DAAs. LS was measured using a FibroScan instrument (Echosens, France) at baseline and 24 weeks after the end of treatment. We didn’t performe LSM on treatment believing that changes occurring during therapy were mainly attributable to the biochemical response rather than to a possible improvement in fibrosis. Only those measurements with at least ten successful acquisitions, with a SR of at least 60% and an interquartile range lower than 30%, were classified as valid and taken into consideration for statistical evaluation. According to this criteria, LSM wasn’t reliable in 6 patients, who were excluded from the final analysis. HCVRNA was assessed monthly during treatment since the 4th week of therapy and at 12 wks and 24 wks follow up.

Results: 64% patients were male. The majority (81%) were well compensated cirrhotics; the median CP score was 5 [95% IC: 5-5] and the median Meld was 8 [95% IC: 7-8]. Only one patient was coinfected HCV-HIV. The median HCVRNA at baseline was 5.88 log [95% IC: 5.73–6.22]. The median baseline values of LS in overall population were 18.6 Kpa [95% IC:15.3–21.3]; not surprisingly median LS was significantly higher in cirrhotics (21.3 Kpa;95% IC: 18-25.1) vs non cirrhotics (10.2 Kpa; 95% IC: 8-15.3), p =0.0001.
All pts but one achieved virological response at 12 and 24 weeks of follow up with a SVR rate of 98.5%. The median values of liver stiffness 24 weeks after treatment were 12.6 KPa [95% IC: 10.4–16.2], significantly lower compared to baseline (p = 0.002). The decrease in LSM was statistically significant also dividing the population for the presence/absence of cirrhosis (p= 0.002 and p=0.02, respectively).

**Conclusions:** We observed a significant decrease in non-invasive fibrosis assessment measured by FibroScan in patients receiving successful DAA treatment. These initial results will be reassessed on long term follow up and need to be histologically confirmed by liver biopsy in the future.

**Disclosure of Interest:** None Declared
Screen 5: YI-MP-213

SURVIVAL OF APOPTOSIS-PRIMED ACTIVATED HEPATIC FIBROBLASTS IS BCL-XL DEPENDENT

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Introduction: Activated stromal fibroblasts (ASF) are the main stromal cell population in liver fibrosis and desmoplastic liver cancers such as cholangiocarcinoma. These cells are drivers of fibrosis development and have been implicated in tumorigenesis. ASF have acquired an activated phenotype with increased sensitivity to apoptotic stimuli. This process is called mitochondrial priming since apoptosis is mediated via Bcl-2 protein-induced permeabilization of the outer mitochondrial membrane. Targeting of these Bcl-2 proteins could be explored for novel therapies. However, the molecular mechanism underlying fibroblast activation and mitochondrial priming in the liver is not well understood. Thus, our aim was to investigate these mechanisms, particularly pro- and anti-apoptotic Bcl-2 proteins involved in mitochondrial priming.

Material and Methods: For in vitro studies, human and mouse fibroblasts were either treated with platelet-derived growth factor (PDGF) alone or in combination with different pro-apoptotic BH3 mimetics. Fibroblasts were examined by qRT-PCR, Western blot and immunofluorescence microscopy. The MDR2-/- mouse model was employed for in vivo studies.

Results: PDGF-activated fibroblasts exhibit an altered Bcl-2 profile, e.g. downregulation of Bcl-2 and upregulation of Bcl-xL mRNA. Apoptosis induction with different BH3 mimetics reveals that survival of activated fibroblasts is specifically Bcl-xL-dependent. Treatment of MDR2-/- mice with pro-apoptotic BH3 mimetic (e.g. navitoclax) reduces liver fibrosis.

Conclusions: Apoptotic priming of ASF can be induced by PDGF and is mediated by changes in the Bcl-2 protein profile. Specific Bcl-xL inhibition causes selective apoptosis in ASF. This provides a potential therapeutic strategy for fibrosis and desmoplastic cancers.

Disclosure of Interest: None Declared
Screen 6: MP-202

THE CHANGE OF LIVER AND SPLEEN STIFFNESS MEASURED BY SHEAR-WAVE ELASTOGRAPHY AFTER TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT PREDICTS OUTCOME

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Introduction: Shear-wave elastography (SWE) of the liver might be a promising tool to predict portal hypertension.

Aims: The aim of our prospective study was to investigate the role of liver and spleen SWE and its evolution in patients receiving a transjugular intrahepatic portosystemic shunt (TIPS) insertion.

Material and Methods: We included 73 patients receiving TIPS for prevention of rebleeding and/or therapy-refractory ascites. Hemodynamic parameters were assessed before TIPS and after TIPS insertion. SWE of liver and spleen, as well as clinical and biochemical parameters were assessed before TIPS, seven days and 6 weeks after TIPS.

Results: Liver SWE (L-SWE) and spleen SWE (S-SWE) decreased after TIPS. However, in subgroup analysis one third showed an increase in L-SWE before and seven days after TIPS. The increase in L-SWE was identified as independent predictor of survival. While L-SWE difference showed no significant correlation, S-SWE difference correlated significantly with change of portal pressure and pressure gradient.

Conclusions: In conclusion, this study demonstrates for the first time that change in L-SWE might also be of therapeutic value in patient with liver cirrhosis receiving TIPS. By contrast S-SWE change might be suitable as TIPS follow-up parameter.

Disclosure of Interest: None Declared
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