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OC-01

MICROPARTICLES RELEASED AS A CONSEQUENCE OF LIPID-INDUCED TOXICITY PROMOTE NLRP3 INFLAMMASOME ACTIVATION IN HEPG2 CELLS

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Introduction: Hepatocytes or HepG2 cells overloaded with saturated lipo-toxic free fatty acids, a condition that mimick lipid accumulation occurring in the liver in some forms of steatohepatitis, have been recently reported to release proangiogenic microparticles (MPs) in a caspase 3-dependent manner, an event which occurs also in vivo and may have a role in the pathogenesis of NAFLD/NASH [1].

Aims: In the present study we investigated whether MPs released from fat-laden cells may affect in a paracrine way NLRP3 inflammasome, which is known to be activated in vivo in NAFLD/NASH [1].

Methods: MPs were collected and purified as released by fat-laden HepG2 (i.e., HepG2 exposed for 24 h to 0.25 mM palmitic acid or PA), as recently described [1]. HepG2 resting cells were then incubated (15 min–24 h) with MPs, LPS (100 ng/mL–1 μg/mL or PA (150–500 μM), the latter known to induce NLRP3 inflammasome in hepatocytes. Expression of NLRP3, pro-caspase and cleaved caspase 1, pro-IL-1 and cleaved IL-1β was evaluated by Western blot analysis in cell lysates, whereas ELISA assays were used to measure IL-1β and IL-18 levels released by resting HepG2.

Results: MPs induced a time-dependent increase in NLRP3 expression in resting HepG2 cells starting from 6 h and then reaching a plateau at 16–24 h, with a kinetics that overlapped the one exerted by PA and was delayed as compared to LPS (1–3 h). Interestingly, both MPs and PA, but not LPS, significantly induced caspase-1 activation and consequent release of IL-1β and IL-18 in a time-dependent manner.

Conclusions: Fat-laden cells, by releasing MPs in a paracrine way, can efficiently trigger inflammasome activation in surrounding hepatic cells, thus identifying an additional new molecular mechanism of inflammation in NASH pathogenesis.

Reference

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OC-02

PRO-INFLAMMATORY AND PRO-FIBROGENIC ACTIONS OF THE HIV-ENVELOPE PROTEIN GP120 ARE MEDIATED BY INFLAMMASOME ACTIVATION AND MIR29B

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Introduction/aim: Patients with HCV/HIV co-infection show faster progression of liver fibrosis, in part associated with miRNA dysregulation and more severe inflammation. The HIV protein gp120 modulates directional migration and expression of profibrogenic cytokines in hepatic stellate cells (HSC), through engagement of the chemokine receptor CCR5. The NALP3 inflammasome is a critical pathway in the generation of proinflammatory signals during liver injury. Aim of this study was to evaluate the role of miRNAs and inflammasome activation in mediating the effect HSC.

Methods: HSC were isolated from normal human liver tissue. Inflammasome complex gene expression was measured by qRT-PCR. Levels of mature IL-1β were assayed by ELISA. miRNA expression was evaluated via RT-PCR. miR-29b mimic was transfected using Amaxa. Blood mononuclear cells (MNCs) were isolated from healthy volunteers.

Results: HSCs exposed to M-tropic gp120 (CN54) showed a time-dependent up-regulation of Pycard, NALP3, Caspase-1 and IL-1β. ELISA showed increased levels of mature IL-1β in the supernatants of gp120-stimulated cell. Pre-incubation of HSC with neutralizing anti-CCR5 antibody reduced gp120-mediated IL-1β production, showing that this receptor is required for activation
of the inflammasome complex by gp120. Similar results were obtained in MNC where a CCR5 receptor antagonist blocked inflammasome activation. gp120 was found to modulate the expression of different miRNAs, and in particular to downregulate mir29b in HSC. Transfection of a miR-29b mimic in HSC blocked the ability of gp120 to induce procollagen-1 expression.

Conclusions: HIV-gp120 mediates inflammasome activation through engagement of CCR5, and regulates procollagen-1 production via down-regulation of miR-29b.

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OC-03
HUMAN CHOLANGIOCARCINOMA (CCA) AND CCA CANCER STEM CELLS (CSCs) ARE HIGHLY SENSITIVE TO THE ANTIPROLIFERATIVE EFFECTS OF PI3-KINASE INHIBITORS
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CCA is highly resistant to chemotherapeutics where resistance is conferred by cancer stem cells (CSCs). We evaluated the in vitro sensitivity of human CCA cells and CSC subpopulations (CD90+/CD13+, CD90+/CD13−) to PI3-kinase/AKT inhibitors, NVP-BEZ235, MK2206, MEK1/2 inhibitor, selumetinib and LY2940680; tyrosin-kinase inhibitor, imatinib mesylate, genistein; aminopeptidase-N inhibitor, bestatin (anti-CD13) should be preferred for treatment of Mixed-CCA subtype. Remarkably, we identified a PI3-kinase inhibitor, NVP-BEZ235, that in vitro exerts marked antiproliferative effect against both Mucin- and Mixed-CCA and their CSCs.

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OC-04
MERTK RS4374383 AA GENOTYPE IS ASSOCIATED WITH A LOWER PREVALENCE OF SEVERE HEPATIC STEATOSIS IN NON-ALCOHOLIC FATTY LIVER DISEASE
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Background aims: MERTK is a tumor-associated macrophage receptor with a key role in efferocytosis and in immune response, and MERTK variants have been associated with fibrosis severity at genome wide level in chronic hepatitis C. We aimed to assess whether rs4374383 MERTK, with and without stratification for PNPLA3 genotype, was associated with the severity of histological features in patients with biopsy-proven NAFLD.

Methods: In 651 consecutive NAFLD patients (244 from Sicily, and 407 from Northern Italy), we assessed anthropometric, biochemical and metabolic features; liver biopsy was scored according to Kleiner. As controls, we evaluated 168 patients without clinical or histological evidence of steatosis. PNPLA3 rs738409 C>G and MERTK rs4374383 A>G SNPs were also assessed.

Results: MERTK rs4374383 A>G SNP distribution was similar in cases compared to controls (p = 0.99). In the entire cohort, MERTK AA genotype (OR 0.25, 95% CI 0.10–0.58, p = 0.001) was independently associated with severe steatosis together with PNPLA3 GG status (OR 2.18, 95% CI 1.32–3.59, p = 0.002). In the high-risk group of PNPLA3 GG patients, severe steatosis was observed in none patients with MERTK AA (0/11) compared to 39% (33/84) with MERTK GG/GA genotype (p = 0.01). The presence of fibrosis >F1 was independently linked to MERTK AA genotype in Sicilian cohort only (OR 0.28; 95% CI 0.11–0.69, p = 0.006), but not in the Northern Italy and in the entire cohorts. However, when excluding subjects with BMI >40 kg/m2 from the entire cohort F2-F4 fibrosis was observed in 19.2% patients with MERTK AA compared to 30.3% with MERTK GG/GA (p = 0.04), being this data confirmed at multivariate analysis (OR 0.44; 95% CI 0.21–0.89, p = 0.02).

Conclusions: MERTK AA genotype is protective against severe steatosis in patients with NAFLD, especially in those at high risk because of PNPLA3 GG genotype, while its effect on liver fibrosis needs to be further investigated.

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OC-05

**ACTIVATION OF THE DEVELOPMENTAL PATHWAY NGN-3/MIR-7A REGULATES CHOLANGIOCYTES PROLIFERATION IN RESPONSE TO INJURY**

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**Introduction and aim:** The activation of the biliary stem-cell signaling pathway Hes-1/PDX-1 in mature cholangiocytes determines cell proliferation. Neurogenin-3 (Ng-n-3) is required for pancreas development and for ductal cell neogenesis. PDX-1-dependent activation of Ng-n-3 initiates the differentiation program, by inducing microRNA (miR)-7 expression. We aimed to verify whether Ng-n-3 regulates cholangiocyte proliferation.

**Methods:** Expression levels of Ng-n-3 and miR-7 isoforms were tested in cholangiocytes from normal and cholestatic livers. Ng-n-3 was knocked down in vitro by siRNA. In vivo, wild type (WT) and Ng-n-3 heterozygous (+/-) mice were subjected to Bile Duct Ligation (BDL) for 2 weeks.

**Results:** In the liver, Ng-n-3 is expressed in cholangiocytes of mice subjected to BDL and of patients affected by PSC, but not in normal conditions. Expression of miR-7a-1 and miR-7a-2 isoforms, but not miR-7b, was increased in BDL cholangiocytes as compared to normal ones. In vitro, Ng-n-3 siRNA neutralized the increases in cell proliferation and in the expression of IGF-1 (a pro-proliferative effector) and miR-7a, but not of PDX-1 or VEGF, observed after exposure to FBS or exendin-4. Anti-sense miR-7 neutralized the effects of FBS or exendin-4 induced increases in cell proliferation but not in PDX-1 and Ng-n-3 synthesis. In vivo, increases in bile duct mass and collagen deposition induced by BDL were significantly reduced in Ng-n-3+/- mice.

**Conclusions:** Ng-n-3-dependent activation of miR-7a is a determinant of cholangiocyte proliferation. These findings indicate that the re-acquisition of a molecular profile typical of organ development is essential for the biological response to injury by mature cholangiocytes.

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OC-06

**NON-INVASIVE SCORE SYSTEM FOR FIBROSIS (NISF) A NEW STAGING MODEL COMBINING BIOCHEMICAL, ELASTOGRAPHIC AND ULTRASOUND DATA IN CHRONIC LIVER DISEASE**

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**Introduction:** Accurate and widely available methods for non-invasive assessment of liver fibrosis are increasingly needed in clinical practice.

**Aims:** To elaborate a Non-Invasive Score system for Fibrosis (NISF) based on combination of biochemical, elastographic and ultrasound data. To explore its diagnostic performance in chronic viral hepatitis (CH) and Non-Alcoholic Fatty Liver Disease (NAFLD). To compare NISF accuracy versus Fibroscan.

**Materials and methods:** Overall 141 patients undergoing liver biopsy were enrolled (83 CH, 58 NAFLD). Clinical, biochemical, elastographic and ultrasound parameters (assessed by 2 blinded operators) were collected as potential predictors of fibrosis. Candidate predictors with good inter-observer agreement and correlation to histologic stage were selected and combined into 2 algorithms (NISF) to predict fibrosis in CH and NAFLD. Predicted fibrosis stages (F0-1, F2, F3-4 and 2 for NAFLD: F0-1, F2-3-4) were compared to Metavir/Brunt histological score.

**Results:** The 2 resulting algorithms can be processed by a calculator as a mathematical formula or graphically displayed as nomograms.

CH-NISF includes 6 parameters: Bluntness of liver edges, irregularity of left lobe surface, segment 4 diameter, liver stiffness, platelet count, ALT values. CH-NISF agrees with liver biopsy in 80%, 62.5% and 79% of F0-1, F2 and F3-4 stages, respectively. The diagnostic accuracy for discrimination of F3-4 vs F0-1, F2 vs F0-1 and F3-4 vs F2 is better for CH-NISF (AUCs of 0.95, 0.83 and 0.92, respectively) compared to Fibroscan alone (0.92, 0.57 and 0.79, respectively).

NAFLD-NISF includes 3 parameters: Liver stiffness, platelet count and AST levels. For discrimination of F0-1 versus F2-3-4 stages, NAFLD-NISF and Fibroscan show similar diagnostic accuracy (0.86 for NAFLD-NISF vs 0.81 for Fibroscan).

**Conclusions:** CH-NISF can be proposed as an easily-available staging tool, superior to Fibroscan alone in predicting histologic fibrosis, especially in intermediate stages. Further evaluations are needed to improve NISF accuracy in NAFLD.

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OC-07

**EPICARDIAL FAT THICKNESS (EAT) IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) IS ASSOCIATED WITH MARKERS OF CARDIOVASCULAR DAMAGE AND SEVERITY OF STEATOSIS**

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**Introduction:** Epicardial adipose tissue (EAT), measured by echocardiography, and proposed as a new index of cardiac and visceral adiposity, has been implicated in the pathogenesis of coronary atherosclerosis. EAT was found increased in patients with metabolic syndrome, of which non-alcoholic fatty liver disease (NAFLD) represents the hepatic manifestation. Aim of this study was to assess in patients with NAFLD and in matched healthy control (a) threshold values of EAT thickness (b) association with the anthropometric, metabolic and clinical parameters (c) association with early atherosclerotic vascular damage, evaluated by carotid
intima–media thickness (cIMT) and (d) association with liver damage.

**Materials and methods:** A cohort of 88 subjects matched 1:1 for sex and age. In all patients EAT thickness was transthoracic echocardiogram, cIMT by ecocolordoppler, complete anthropometric, clinical and biochemical data were evaluated.

**Results:** Patients with NAFLD had significantly higher EAT thickness than controls (5.4 ± 2.7 vs 2.8 ± 2.4 mm). Considering as increased EAT thickness a value >2.8 mm (median of controls) we evaluated variables associated with increased EAT. At univariate analysis BMI (p = 0.02), hypertension (p = 0.04), fasting glucose (p = 0.0003), and ALT (p = 0.05), were significantly associated with increased EAT thickness. A significant association between EAT and cIMT and diastolic echographic alteration (both p = 0.01) was found. At multivariate analysis NAFLD was the strongest independent variable associated with increased EAT (OR 5.37, 95% CI 1.75–18.1, p = 0.003). EAT thickness increased with severity of ultrasonographic steatosis (p for trends = 0.002), and with histological grade of steatosis, evaluated in 43 patients (p = 0.01).

In conclusion, EAT thickness is significantly higher in NAFLD patients and it is associated with markers of cardiovascular damage and severity of steatosis.

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**OC-08**

**ADULT HUMAN BILIARY TREE STEM CELLS (hBTSCs) ARE EFFICIENTLY REPROGRAMMED TO FUNCTIONAL INSULIN-SECRETING β-PANCREATIC ISLET CELLS BY A NEWLY SYNTHESIZED HUMAN PDX1 PEPTIDE**

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**Introduction:** Liver cells have been reprogrammed to pancreatic fate by adenoviral transfection of three-gene cocktail containing pancreatic and duodenal homeobox 1 (PDX1) in diabetic mice. The aim of this study was to explore a protein-based strategy to induce differentiation of human liver stem cells towards β-pancreatic fate.

**Materials and methods:** A plasmid contains the entire sequence of the human PDX1 have been expressed in E. coli. Epithelial-Cell-Adhesion-Molecule positive stem/progenitor cells were immunoselected from human biliary tree (hBTSCs) discharged from adult donor livers and transferred into Kubota’s Medium (KM) added with 0.1/0.5 μM Pdx1, or into conditioned medium tailored for pancreatic islets.

**Results:** The western blotting (WB) and spectroscopy analyses confirmed the efficient production of a purified His-tag human Pdx1 protein (43 kDa). The cell viability remained stable in cultures exposed to 0.1 μM Pdx1. WB analysis confirmed the effective cell internalization of the stable Pdx1. hBTSC cultures exposed to Pdx1 (0.1 μM) compared with cells in KM show ed significant increase of insulin gene expression by RT-PCR (>2.5-folds; N = 6; p < 0.05), and the appearance of islet-like structures constituted by cells intensely positive for insulin and Pdx1 by immunofluorescence (KM, N = 6; p < 0.05). When Pdx1-induced islet-like structures (N = 7; p < 0.05) were challenged with high (22 mM) glucose concentrations, the stimulated c-peptide secretion was similar to that measured in cultures of hBTSCs differentiated in islet cells by conditioned medium tailored (by hormone mixtures) for pancreatic differentiation.

**Conclusions:** The newly synthesized human Pdx1 peptide efficiently reprogrammed hBTSCs to functional β-pancreatic islet cells with important implications for the regenerative medicine of pancreatic diseases and diabetes.

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**OC-09**

**STEM CELL NICHE AND MACROPHAGES IN HUMAN CHOLANGIOCARCINOMA**

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**Introduction:** Tumorigeneicity is modulated by inflammatory microenvironment mainly composed by infiltrating macrophages (MØ). Neoplastic evolution is associated with MØ-phenotypic switch from M1 (chronic inflammation sites) to M2 subtype (established tumors).

**Aims:** Our study aims to highlight the dynamic interactions between stem-like tumor initiating cells (TICs) and MØ in human cholangiocarcinoma (CCA).

**Materials and methods:** Stem-like compartment was identified by sphere forming assay in both transformed (SG231, CCLP-1, HuCCT-1, TFK-1) and normal cholangiocytes (H69, HiBECs). Impact of MØ, M1 and M2 on CCA-TICs was tested by sphere forming assay and global gene expression profiling. Additionally, effect of conditioned media derived from CCA sphere (Sp-CM) and monolayer (Mon-CM) was tested on monocyte (CD14+) differentiation and MØ polarization by FACS analysis and RT-PCR ARRAY.

**Results:** Only CCA cells were able to form 3D spheres compare to normal counterpart. Strikingly, normal cholangiocytes acquired spheres forming capacity in presence of M2, whereas CCAs showed a considerable increase of this capability. Furthermore, CD14+ cocultured with Sp-CM, assumed an elongated shape and did not show any of typical differentiation (CD115) or polarization (CD80, CD206, CD209) markers, indicating a “new” phenotype. Notably, Sp-CM played a more effective impact on MØ as revealed by the expression of CD80 suggesting a “mild M1-like” subtype. Conversely, CD14+ or MØ cultured in presence of Mon-CM expressed only CD206 representing a “mild M2-like” features.

**Conclusions:** Our results suggested a prominent interplay between CCA and TICs and macrophages. The described model might allow the development of alternative therapeutic strategies for CCA.

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OC-10

SYMPTOMATIC ONSET IDENTIFIES A MORE AGGRESSIVE SUBSET OF PRIMARY BILIARY CIRRHOSIS


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Introduction & aims: Primary biliary cirrhosis (PBC) is usually diagnosed during the asymptomatic phase, however a proportion of patients still present with symptoms such as fatigue and/or pruritus. We compared biochemical, histological and immunological features of symptomatic and asymptomatic patients to see whether the different clinical presentation may eventually have an impact on disease progression.

Patients and methods: A cohort of 216 patients with PBC referred to us between 1997 and 2007 has been analysed and compared according to the symptomatic (fatigue and/or pruritus) or asymptomatic presentation. Clinical, biochemical, histological and immunological feature at diagnosis, response to ursodeoxycholic acid and progression of the disorder were evaluated after a mean follow up of 81 ± 25 months.

Results: At diagnosis symptomatic patients were significantly younger (mean age 49 ± 12 years versus 55 ± 12, p = 0.003) and with more pronounced biochemical activity, as indicated by higher alkaline phosphatase (mean × upper normal limit 2.93 ± 2 vs 2.12, p = 0.002) and aminotransferase (mean × upper normal limit 1.92 ± 1 vs 1.47 ± 1.2, p = 0.014) levels, whereas histological stage and autoantibody profile were similar. Symptomatic patients were less likely to respond to ursodeoxycholic acid therapy (61% vs 81.5%, p = 0.005) and developed more often cirrhosis and its complications (31% versus 13%, p = 0.005) after a comparable follow up.

Conclusions: Symptomatic PBC at onset consists of a peculiar subset of younger patients, particularly active biochemically, less responsive to ursodeoxycholic acid treatment, and more often evolving to cirrhosis and its complications. Innovative therapeutic strategies are urgently needed for these PBC patients.

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OC-11

A NOVEL SIMPLIFIED CHILD–TURCOTTE–PUGH SCORE FOR PROGNOSTIC ASSESSMENT AND THERAPEUTIC DECISION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Background: In patients with hepatocellular carcinoma (HCC), Child Turcotte Pugh score (CTP) is mainly used as a three-classes-staging (A, B, and C) and included in this form in the Barcelona Clinic Liver Cancer staging and treatment algorithm.

Aim: To propose and validate a higher performance version of CTP (Na-CTP).

Methods: Derivation data were obtained from the ITA.LI.CA. (Italian Liver Cancer) database (n = 5134, period 1986–2012). We recruited 1720 consecutive HCC patients (period 2000–2012) with known liver cirrhosis, undergoing non-transplant therapies and with complete data to assess prognostic ability of CPT, model–for-end-stage–liver disease (MELD) score, and MELD-Na score.

A series of 2651 consecutive HCC patients from Taipei, Taiwan (period 2002–2012) were used for external validation. A novel score (Na-CPT) was developed in the derivation cohort from a multivariate Cox model including CPT variables plus serum sodium. Internal (cross validation) and external validation of the model was performed. Discrimination and calibration of Na-CPT were compared against existing models.

Results: Evaluating the behaviour of CPT variables included in a Cox model, we developed the Na-CPT (score 6–13) based on albumin as in the original model and on the dichotomy of the following covariates: presence/absence of ascites and encephalopathy, INR > 2.3, bilirubin > 2, sodium < 135. Although simplified, in the derivation cohort Na-CPT showed the best discrimination power and calibration with respect to other models. In cross-validation, c-statistics and likelihood ratio tests remained largely unchanged. In the validation cohort, discrimination ability and calibration were not inferior to CPT and remained largely better than MELD and MELD-Na.

Conclusions: We developed a novel Na-CPT, easy to apply in clinical practice, and with a strong prognostic impact in two large western and eastern populations. This new score, introducing a wider range of prognostic categories than the traditional three-CPT-classes, could help in selecting the most suitable therapeutic choice for each HCC patient.

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OC-12

THROMBELASTOGRAPHY (TEG) DECREASES BLOOD PRODUCTS REQUIREMENT BEFORE INVASIVE PROCEDURES IN CIRRHOTIC PATIENTS WITH COAGULATION TESTS DERANGEMENT. A RANDOMIZED CONTROLLED TRIAL

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Background: Although cirrhosis can be characterized by a thrombophylic state, bleeding is the most feared event in end-stage liver disease. Therefore, blood products are largely used before invasive procedures and the risk of bleeding is still assessed by conventional coagulation tests (platelets count and INR). TEG is able to detect patterns of hypo-hypercoagulability. Aim was to evaluate the efficacy of TEG before invasive procedure as a guide for hemoderivatives transfusion in cirrhotics.

Methods: Cirrhotics with coagulation disorders (INR > 1.8 and/or PLTs < 50 × 10^3 /mmc) undergoing invasive procedures were eligible. Exclusion criteria: bleeding, thrombosis, anticoagulants.
or antiaggregants, sepsis and hemodialysis. Patients were randomly allocated either to TEG group (TEG-G), receiving fresh frozen plasma (FFP 10 ml/kg) in case of R > 40 mm and/or platelets (PLTs 1 unit/10 kg) for MA <30 mm before procedure, or to Per-Protocol Group (PPG), receiving PLTs and/or FFP before procedures according to internal guidelines.

Results: 48 patients were enrolled so far, 25 in the TEG-G and 24 in the PPG. No differences at baseline parameters were found, in particular age (57 ± 10.9 vs 60.7 ± 12.4), INR (2.02 ± 0.65 vs 2.17 ± 0.73) or platelets (57.6 ± 34.9 vs 67.5 ± 43.5 × 10^9/mmc), neither the type of procedures performed (p > 0.05). Every subject in the PPG received transfusions as compared to 6 in the TEG-G (100% vs 21.4%, p = 0.000). In the PPG 15 patients (62.5%) required FFP, 5 (20.8%) PLTs, and 4 (16.6%) both PLTs and FFP. In the TEG-G none receive FFP alone, 2 needed PLTs (8%), 4 both PLTs and FFP (16%). No post-procedural bleeding or complications occurred in both groups but a transfusion-related reaction in the PPG. Survival was similar in both groups (LR p = 0.78). Mean transfusion cost for the TEG-G was 188 €/patient (including TEG cost), 297 €/patient for the PPG (p = 0.000).

Conclusion: TEG is safe and effective to guide transfusion before invasive procedures reducing transfusion requirement, risk of transfusion-related side effects, and medical costs.

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OC-13
CASPASE-3 TARGETING: A FURTHER TILE SUSTAINING THE ANTI-APOPTOTIC ROLE OF miR-221 IN HEPATOCELLULAR CARCINOMA
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Introduction: MicroRNA-221 (miR-221) is over-expressed in several cancer types including hepatocellular carcinoma (HCC). It was previously demonstrated that miR-221 reduced apoptotic cell death through the direct targeting of pro-apoptotic genes, such as BMF and PUMA. MiR-221 oncogenic role has been proved by our group in a liver-specific miR-221 transgenic model, suggesting antagoniMiR-221 as a possible therapeutic tool for the treatment of HCC.

Aim: This study aims to further characterize miR-221 involvement in the regulation of apoptotic cell death and response to treatments through the direct regulation of caspase-3 expression.

Methods: An in vitro functional analysis was used to investigate miR-221 modulation of caspase-3 expression in HCC cell lines. Luciferase-reporter assay was performed to evaluate the interaction between miR-221 and its complementary binding site in caspase-3 mRNA. Chemiluminescent assays, FACS and Western blot analysis were performed to evaluate apoptotic cell death in miR-221 or anti-miR-221 transfected HCC cells following Doxorubicin treatment. Real Time PCR and Western blot analysis were employed to analyse miR-221 and caspase-3 expression in liver tissues of miR-221 transgenic model.

Results: Western blot analysis and luciferase reporter assay showed a direct regulation of caspase-3 target by miR-221 in different HCC cell lines. Annexin-V/FACS analysis displayed an increase of apoptotic cell death in miR-221 silenced Hep3B and SNU449 cells following Doxorubicin challenge. In line with this data, an increase of caspase activity and pro-apoptotic genes expression was observed in the same settings. An ex vivo analysis showed a significant decrease of caspase-3 protein levels in liver tissues of miR-221 transgenic mice with respect to wild type control animals.

Conclusions: MiR-221 regulates apoptotic cell death of HCC cells through the simultaneous inhibition of caspase-3 protein and other pro-apoptotic molecules. Our data provide an additional molecular rationale for miR-221 silencing as a novel therapeutic strategy for the treatment of advanced HCCs.

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OC-14
REINFUSION OF HIGHLY PURIFIED CD133+ STEM CELLS IN PATIENTS WITH END-STAGE LIVER DISEASE (ESLD): FINAL RESULTS OF A PHASE I CLINICAL TRIAL
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Introduction: Previous studies have shown that bone marrow stem cells (BMSCs) contribute to liver regeneration after tissue injury.

Aim: The aim of the present study was to evaluate the feasibility and safety of intrahepatic reinfusion of an increasing number of highly purified CD133+ BMSCs in patients with ESLD.

Materials and methods results: Patients with MELD score ≥17 were enrolled in the study. Autologous CD133+ cells, mobilized after G-CSF administration, were collected from peripheral blood by standard volume leukapheresis and selected with Clin-iMacs device. Finally, an increasing number of highly purified CD133+ cells were reinfused through the hepatic artery starting from 5 × 10^6/kg/BW up to 1 × 10^6/kg/BW. Vascular Endothelial Growth Factor (0.97 ± 0.97 vs 13 ± 8.69 pg/ml, p < 0.001) and Vascular Endothelial Growth Factor (0 ± 0.97 vs 13 ± 8.69 pg/ml, p < 0.001) was recorded.

Conclusions: CD133+ BMSCs reinfusion in patients with ESLD is feasible and safe. The improvement of liver function seen during
OUTCOME INDICATORS IN LIVER CIRRHOSIS: APPLICATION OF VALUE-BASED MEDICINE IN A LARGE MULTICENTER STUDY (VBMH STUDY)

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Introduction: Liver Cirrhosis (LC) is responsible for high morbidity, mortality, and costs, with increasing need to improve quality of care. Aim of our study (Value Based Medicine in Hepatology Study, VBMH) was to identify outcome indicators (OIs) able to measure quality in liver diseases, including compensated (CC) and decompensated (DC) LC.

Methods: A panel of hepatologists identified a list of 7 OIs for LC according to experience and published evidence, using a modified Delphi method and a standard 9-point RAND appropriateness scale. Then, these 7 OIs were tested in clinical practice in a prospective multicenter observational study involving three tertiary centers in Italy, using a web-based electronic medical record. 1751 LC patients were enrolled in 18 months: 1004 CC and 747 DC. 92% of patients had at least two consultations in 17 months median follow-up.

Results: Annual rate of decompensation in CC was 12% (OI#1). Annual incidence of 1st variceal bleeding (VB) was 2% for low-risk and 3% for high-risk varices (OI#2) indicating a significant success of primary prophylaxis. Annual incidence of HCC in CC was 4.3%, with 81% patients found at early stage (OI#3), underlining the accuracy of oncologic surveillance. One-year survival after the first decompensation episode (ascites in 74% of cases) was 96%, 81% and 59% stratified for CPT score A, B, C respectively (OI#4), and 94%, 56% stratified for MELD below or above 15 (OI#5). 4% of DC patients had an episode of VB with 90% survival after 6 weeks, and 33% recurrence (OI#6). Similarly, 3% of DC patients had spontaneous bacterial peritonitis with 6 weeks survival of 86% and 13% recurrence (OI#7).

Conclusions: The value-based OIs generated in this study performed well in a large cohort of consecutive patients, and could represent a reference tool for healthcare providers to improve care in LC.

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HIGH DONOR-AGE VS HIGH MELD IN LIVER TX. WHICH IS THE MOST EFFECTIVE APPROACH? EVIDENCES FROM THE ITALY-US D-MELD STUDY

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Donor-2-Recipient match (D2Rm) remains subject to debate in liver transplantation. A comparative analysis of D2Rm between Italy and US has not been performed. D-MELD (product of donor-age × biochemical MELD) has been used to identify poor matches. Individual prediction of prognosis using D-MELD can be achieved using specifically developed applications (www.D-MELD.com; i-phone/i-pad D-MELD app).

To investigate differences in D2Rm, UNOS and Italian D-MELD databases (2002–2009) were merged. Pediatric cases, multi-organ Tx, SPLITs, liver donors, national share cases and DCDs were excluded. There were 36,795 cases (US = 31,569, 137 Centers; ITALY = 5226, 21 Centers). Donor age (X: IQR) was lower in US than in Italy (43, 25–55 vs 56, 40–68), MELD was higher in US than in Italy (20, 14–28 vs 15, 11–21). Median D-MELD was similar: US = 759 (462–1175); ITALY 783 (520–1163). Low D-MELD (<338) was prevalent in Italy (20, 14–28 vs 15, 11–21). More D-MELD quantified by D-MELD remains a strong predictor of PS (p < 0.001), Cholestasis (HR 1.5, p < 0.001), Portal thrombosis (HR 1.3, p < 0.001), HBV (HR 0.8, p = 0.003), Acute liver failure (HR 1.8, p = 0.001), HCV (HR 1.5, p < 0.001), HBV (HR 0.8, p = 0.003), Cholestasis (HR 1.5, p < 0.001), Low D-MELD vs Intermediate D-MELD (HR 0.7, p < 0.001), High D-MELD vs Intermediate D-MELD (HR 1.8, p < 0.001), Portal thrombosis (HR 1.3, p < 0.001), Italy vs US (HR 0.9, p = 0.007); Chi² = 934. US vs Italy difference in adjusted PS was significant. D2Rm quantified by D-MELD remains a strong predictor of PS in Italy and US. In Italy the use of elderly grafts is advanced leading to better adjusted survival figures (UTILITY approach). In US larger number of severely decompensated pts are transplanted (URGENCY approach).

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OUTCOME INDICATORS IN LIVER CIRRHOSIS: APPLICATION OF VALUE-BASED MEDICINE IN A LARGE MULTICENTER STUDY (VBMH STUDY)

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OC-17

IMPACT OF MTOR INHIBITION ON EXPRESSION OF IMMUNE REGULATORY MOLECULES BY CIRCULATING DENDRITIC CELLS AND REGULATORY T CELLS IN STABLE LIVER TRANSPLANT PATIENTS

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Introduction: Everolimus, a mammalian target of rapamycin (mTOR) inhibitor, is a ‘tolerance-sparing’ immunosuppressant used in solid organ transplantation. mTOR regulates diverse functions of professional antigen-presenting cells, in particular dendritic cells (DCs), and has important roles in the activation of conventional T cells and the function and proliferation of regulatory T cells (Treg).

Aim: Currently, no data are available concerning the impact of everolimus on DC and Treg in stable liver transplant patients. Therefore, the aim of this pilot study was to analyze peripheral blood DC subsets and Treg in 10 liver transplant patients exposed to everolimus (mTOR).

Materials and methods: Ten patients taking a calcineurin inhibitor (CNI; tacrolimus) served as controls group. Moreover, the Expression of key co-stimulatory and co-regulatory molecules on DC and Treg were examined by flow cytometric analysis.

Results: Our findings show that everolimus-treated liver transplant patients maintain a stable DC subset distribution and phenotype. Thus, expression of co-stimulatory and co-regulatory molecule (CD86, CD83, PD-L1 and ICOS-L) did not differ between the mTOR and CNI groups. Notably, however, expression of the ectonucleotidase CD39 was significantly higher on myeloid DC in patients taking everolimus compared with the CNI. The incidence of Treg did not differ between the two groups, but expression of Programmed death-1 (PD-1) on Treg in the everolimus group was significantly lower compared with controls.

Conclusions: This preliminary study provides new information about how mTOR inhibitor-based immunosuppression may influence peripheral innate and adaptive immune cells in liver transplant patients. Our findings also point out possible new biomarkers of liver graft acceptance which could be monitored after transplantation.

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OC-18

ITALIAN SURVEY ON HBV AND LIVER TRANSPLANTATION IN A COHORT OF 2260 PATIENTS


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* AM and LC shared the production of the survey.
Introduction: HBIG (MONO) alone or combined with NUCs (Combo) is used for preventing post-LT HBV recurrence (PLTHB) and de-novo hepatitis B (DNHB) from anti-HBc + grafts.

Patients and methods: Data from LTs performed in 13 Italian Transplant Centers (TC) or monitored in 11 Outpatients Centers (OC) in the period 1983–2011 (T1) and in 2011 (T2) were analyzed.

Results:

<table>
<thead>
<tr>
<th></th>
<th>LTs total</th>
<th>LTs in HBsAg + patients</th>
<th>LTs with antiHBc + grafts</th>
<th>PLTHB</th>
<th>DNHB</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>10,365</td>
<td>2260 (21% HDV+)</td>
<td>1043 (10%)</td>
<td>3.7%</td>
<td>6%</td>
</tr>
<tr>
<td>T2</td>
<td>689</td>
<td>128</td>
<td>100 (14%)</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Considering PLTHB, 52% of the TC used NUC(s) pre-LT in viremic patients (threshold HBV DNA limit for the treatment >12/200/2000/20,000 IU/ml in 55/9/27/9%). Before activate the listing, HBV DNA <20,000 UI/ml or negative was required in 96% and 35% of HBsAg-positive patients. LTs for HCC in HBsAg-positive patients showed an increasing rate in the last years. In HBsAg-positive patients combo prophylaxis was used lifelong in 90% of the TC, and only 5-24% of them reported NUC(s) or HBIG withdrawal. In recipients of anti-HBc-positive grafts single HBIG, single NUC or combo prophylaxis was used in 0, 58% and 42% of centers, respectively. Intravenous (IV) HBIG formulations were preferred in the early post-LT period (1–3 months) in 70% of the Centers, and intramuscular (IM) HBIG thereafter (86%). Complete adherence to NUCs and 90% to IV HBIG were observed in 80% of the centers. After LT, HBsAg was checked every 7–30 days in the first month 30–90 days thereafter. Anti-HBs titers were aimed to >300 IU/ml early, 100–250 IU/ml during the first year, >100 IU/ml thereafter.

Conclusions: Our survey for the first time depicts the Italian way to anti-HBV prophylaxis, confirming excellent results by the use of heterogeneous policies among centers.

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OC-20

ROLE OF SIALIC-ACID-BINDING IMMUNOGLOBULIN-LIKE LECTIN-7 (SIGLEC-7) AS BIOMARKER OF LIVER DISEASE SEVERITY IN CHRONIC HCV INFECTION

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Introduction/aim: Siglec-7 (S7) is a NK cell inhibitory receptor that recognizes pathogen expressing sialylated surface glycans and is associated with NK phenotypic and functional abnormalities in HIV-1 infection. We asked whether S7 could bind HCV E2 protein and whether NK-expressed and serum S7 (sS7) correlated with clinical parameters and/or identified dysfunctional NK subsets.

Materials and methods/results: S7 binding to HCVE2 was assessed by flow cytometry using a S7 chimeric protein and HCVE2-conjugated beads. NK-expressed and serum S7 were evaluated in patients with chronic HCV infection (HCVp, n = 169) and healthy donors (HD, n = 167) by flow cytometry and ELISA, respectively. Degranulation and cytokine secretion was determined in S7+ and S7− NK cells after co-culture with K562 cells. S7 exhibited strong binding to HCVE2. The proportion of S7-expressing NK was significantly reduced and, conversely, sS7 levels were increased in HCVp compared with HD (p = 0.002 and p < 0.0001, respectively). ROC analysis of sS7 as predictor of HCV infection showed a AUC of 0.84 (95% CI 0.80–0.89) with specificity and sensitivity of 82.2% and 78.4% (c/o 1450 pg/ml). There was a positive correlation between sS7 and serum AST (p < 0.0001), ALT (p = 0.0002) and liver stiffness by transient elastography (p = 0.01) suggesting a role for S7 in liver injury in HCV infection. S7 negative NK cells produced less TNFα in HCVp than in HD (p = 0.03), identifying the existence of a dysfunctional NK subset in chronic HCV infection.

Conclusions: HCV binds and modulates S7, likely shed from NK cells in HCVp. Serum S7 emerges as a potentially useful biomarker of liver disease severity in chronic hepatitis C.

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OC-21

THE CO-ADMINISTRATION OF TELAPREVIR INCREASES RIBAVIRIN PLASMA AND INTRA-ERYTHROCYTIC CONCENTRATIONS, CAUSING HIGHER ONSET OF ANEMIA

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Introduction: The new standard of care (SOC) for treatment of HCV-1 is the association of Telaprevir (TEL) or Boceprevir (BOC) to Ribavirin (RBV) and Peg-Interferon alfa. Despite the improved efficacy, a higher frequency of hemolytic anemia was observed. Anemia is a typical side effect of RBV.

Aim: Our aim was to investigate the existence of a concentration-dependent interaction between TEL and RBV.

Materials and methods: To evaluate this possible interaction 17 patients treated with SOC were compared to 119 with dual therapy. Moreover, the same comparison was performed in a sub-group of 9 out of 17 patients who were treated 1–2 years before with dual therapy, and recently re-treated with SOC. This comparison provided data without interferences due to the inter-patient variability. RBV plasma and intra-erythrocytic levels and TEL (S and R isomers) plasma concentrations were determined after 4 weeks of therapy with validated chromatographic methods.

Results: No significant differences in weight-based dose of RBV were observed between therapies. In the 9 patients sub-group, both RBV plasma and intra-erythrocytic concentrations were significantly higher during retreatment (p = 0.015 and p = 0.012, respectively). This evidence was confirmed for intra-erythrocytic concentrations in the overall treated patients (p = 0.040). Triple therapy treated patients showed a higher incidence of anemia (88% vs. 37%, p < 0.001). Interestingly, a significant correlation (p = 0.023) emerged between hemoglobin drop and RBV plasma concentration. Moreover, RBV and TEL-S plasma concentrations were significantly (p = 0.008) correlated.

Conclusions: The co-administration of TEL increased RBV concentrations in a concentration-dependent manner, leading to a higher incidence of anemia. This unbiased evidence highlights the need of specific cut-off values for RBV and TEL-S concentrations. These evidences justify the use of Therapeutic Drug Monitoring (TDM) to manage toxicity, guiding the “ongoing” dose modification to maintain patients on therapy.

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HBSAG GENETIC ELEMENTS CRITICAL FOR IMMUNE ESCAPE CORRELATES WITH HBV REACTIVATION UPON IMMUNOSUPPRESSION

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Background: HBV-reactivation is defined as an abrupt reappearance or rise of serum HBV-DNA in patients with resolved or inactive HBV infection (Hoofnagle, 2009). The role of HBsAg genetic diversity in this phenomenon is still anecdotic. Here, we investigate HBsAg genetic signatures underlying immunosuppression-driven HBV-reactivation.

Methods: This study includes 93 HBsAg-sequences from 29 patients with HBV-reactivation triggered by immunosuppressive therapy, and 64 chronically HBV-infected drug-naïve patients as control (all genotype-D). HBsAg ultra-deep sequencing (UDPS) is also performed for 21/29 HBV-reactivating patients.

Results: 55.2% of patients with HBV-reactivation is treated with rituximab for hematologic-malignancies, 24.1% with corticosteroids for auto-immune/inflammatory/neoplastic diseases, and 20.7% with other immunosuppressive-chemotherapeutics. 48.3% of patients experienced HBV-reactivation after completing immunosuppressive-therapy (range: 1–14 months); Among 9 HBV-reactivating patients despite lamivudine-prophylaxis, drug-resistance is detected in 5 patients.

72.4% of HBV-reactivating patients (compared with <1.5% of controls, P<0.001) carries specific HBsAg mutations localized in HBsAg-regions relevant for HBV immune-control. Of the 13 HBsAg-mutations correlated with HBV-reactivation, 5/13 (T118K-P120A-Y134H-S143L-D144E) reside in the a-determinant, and are known to hamper HBsAg-recognition by antibodies; 8/13 (C48G-V96A-M103I-L109I-S171F-L175S-G185E-V190A) are localized in Class-I/II-restricted T-cell epitopes, playing a potential role in HBV-escape from T-mediated response. Furthermore, additional N-linked glycosylation-sites within the a-determinant are found in 24.1% of HBV-reactivating patients, compared with 0% of controls (P<0.001); N-linked glycosylation can mask immunogenic-epitopes, abrogating HBsAg-recognition by antibodies. By UDPS-analysis, 38.1% of HBV-reactivating patients carries minority-mutations in a-determinant, including vaccine-escape T131N-M133I-G145R (intra-patient prevalence:0.1–18.1%), confirming the “immune-escape” viral-phenotype characterizing HBV-reactivation.

Conclusions: HBV-reactivation occurs in a wide variety of immunosuppressive clinical-settings, also after completing immunosuppressive-therapy, and is driven by a complex quasispecies carrying HBsAg-mutations with enhanced-capability to evade immune-response. This underlines the importance of a careful patient-monitoring in all immunosuppressive-settings at reactivation risk and of establishing a prompt and potent therapy in order to prevent HBV-related clinical complications.

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OC-24

EFFICACY AND SAFETY OF BOCEPREVIR-BASED THERAPY IN HCVG1 TREATMENT-EXPERIENCED PATIENTS WITH ADVANCED FIBROSIS/CIRRHOSIS: ITALIAN NPP SURVEY


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Introduction and aims: Efficacy, safety profile and re-assessment of futility rules (FRs) in treatment-experienced patients with HCV-related advanced fibrosis/cirrhosis receiving boceprevir-based triple therapy (BOC) were reported.

Methods: Prospective multicentre national registry including treatment-experienced patients with G1 HCV-related cirrhosis or advanced fibrosis treated with P/R/BOC in the NPP early access program.

Results: From February 2011 to December 2012, 266 patients (68.4% male, mean age 56 yrs, 79.3% G1b, 56.4% F4 metavir, 91% Child A5, 19% with oesophageal varices; 33.5% relapsers, 22.2% partial and 43.6% null responders) were enrolled in 34 sites. Platelets count <100.000 and albumin levels <3.5 g/dl were present in 6.8% and 4.0% of patients, respectively.

Overall, in the ITT analysis, SVR12 rate was 45.9% (55.3% in F3, 38.8% in F4; p = 0.01), while it was 57.3% in relapsers, 48.1% in partial responders and 35.8% in null responders respectively. At multivariate logistic regression analysis the strongest predictors of SVR12 was HCV-RNA undetectable at TW8, (RR, 95% CI: 26.0 (6.99–96.7)). At TW8, either HCV-RNA >1000 UI/mL (RR, 95% CI: 5.59 (1.47–21.3)) or decline of HCV-RNA <3 log (RR, 95% CI: 6.00 (1.28–28.0)) were independently associated with poor response. Anemia was the most frequent adverse event, 52% of grade 2/3, 15% of grade 4. Nine percent of patients required blood transfusion and 50% were ultimately supplemented with EPO. One patient (0.4%) died for MOF at week 6, while 3% of patients developed severe infections or hepatic decompensation.

Conclusions: In treatment-experienced patients with advanced fibrosis/cirrhosis, SVR rate achieved by BOC was satisfactory. Despite the incidence of severe adverse events was lower in comparison to other real-world surveys, the rate of severe anemia remains a major concern. A new early FRs at TW8 enables a safely discontinuation of BOC in patients who are highly unlikely to attain SVR.

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OC-25

EPIDEMIOLOGICAL EVOLUTION OF CHRONIC HEPATITIS DELTA IN ITALY: AN ANALYSIS OF THE MASTER-B COHORT

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2 Azienda Ospedaliera di Parma, Italy
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Introduction: Chronic Hepatitis Delta Virus (HDV) infection showed a rapid decline in Italy over the past two decades, reaching current prevalences between 7 and 9% among HBsAg carriers. Immigration from endemic areas caused a resurgence of HDV infection in some European countries. We analyzed the prevalence of HDV in a cohort of Italian and immigrant persons.

Methods: The Master-B study enrolled 2916 HBsAg positive, consecutive subjects in 77 Italian Centers. The proportion of patients tested for anti-HDV ranged from 0 to 100%. For the purpose of the present analysis we selected those centers with <20% missing for anti-HDV.

Results: We analyzed 1011 patients seen in 22 centers; (716 Italians and 295 immigrants). The overall prevalence of anti-HDV was 87/1011 (8.4%). Patients with anti-HDV were younger (median age 47.7 vs 49.3; p < 0.0001), more frequently had cirrhosis (50.5 vs 25.3%; p < 0.0001) and were positive for anti-HCV (22.7 vs 2.4%; p < 0.0001). No difference was found in gender, presence of HBeAg or HCC. Anti-HDV was detected in 53/716 Italians (7.4%) and in
43/295 immigrants (11.5%; p = 0.036). Italian patients with HDV were older (median age 51.1 vs 36.3; p < 0.0001), more frequently males (83.1 vs 29.4%; p < 0.0001) and with cirrhosis (67.9 vs 23.5%; p < 0.0001). HBeAg was present in 8.5 vs 18.7% and anti-HCV in 27.7 vs 14.3% (both p = 0.18). Laboratory showed higher values (median IU) in Italian patients for ALT (71 vs 46.5), AST (56 vs 38) and GGT (44 vs 24) and lower values for platelet count (129 vs 186 × 10^3/μl) (all p < 0.001). When first seen, 82% of immigrants and 38% of Italians had never been treated.

**Conclusions:** Testing for anti-HDV should be implemented in Italy, since a new wave of patients with HDV coinfection is coming from areas where Delta virus is endemic. There are still barriers for immigrants to access medical care.

http://dx.doi.org/10.1016/j.jldl.2014.01.030

**OC-26**

**EARLY VIROLOGICAL RESPONSE IS THE MAIN PREDICTORS OF VIRAL ERADICATION IN HCV HAEMODIALYSIS PATIENTS TREATED WITH PEGYLATED INTERFERON PLUS RIBAVIRIN**

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**Background and aim:** HCV infection is observed in around 20% of dialysis patients and in allograft recipients and results in a significant morbidity and mortality, especially after transplantation. Therefore, eradication of the virus in those waiting for liver transplant is strongly advised. However, less than 5% of HCV dialysis patients waiting for transplant receive pegylated interferon and ribavirin in view of the poor tolerability. On treatment predictors of viral response which could prompt for increased intervention are strongly claimed from Kidney Disease Improving Global outcome (KDIGO) Society.

The overall SVR was 55.5% (20/36), whereas the drop-out rate was 28%. The only factor associated with SVR identified by univariate logistic regression was early viral response (EVR), defined as undetectable HCV RNA at week 12 after the start of treatment. EVR was obtained in 24 patients (67%). The positive and negative predictive value of EVR was 71 and 80% respectively.

**Conclusions:** Early viral response had a high negative predictive value for SVR in haemodialysis patients with chronic hepatitis C treated with PEG-IFN and ribavirin. If HCV RNA is detected at week 12, treatment should be discontinued due to the low probability of a sustained viral response.

http://dx.doi.org/10.1016/j.jldl.2014.01.031

**OC-27**

**LONG-TERM OUTCOME OF INACTIVE AND LOW VIREMIC HBEAG NEGATIVE ACTIVE CARRIERS: BENIGN ONE DIRECTION TRANSITION TOWARDS SPONTANEOUS HBsAg CLEARANCE**

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2 General Medicine 2 Unit and Gastroenterology Chair, University Hospital of Pisa, Italy

**Introduction:** Studies on the long term outcome of HBV infection in HBeAg negative inactive (IC: HBV-DNA persistently ≤2000 IU/ml) and low viremic active carriers (LV-AC: HBV-DNA persistently ≤20,000 IU/ml) are missing.

**Aim:** We studied prospectively the natural course of HBV infection in a large cohort of these HBV low viremic carriers.

**Materials and methods:** In 113 carriers (genotypes: 112D, 11A, 1 for B, E, F, G, H and 5 undetermined) ALT and HBV-DNA were tested at least every 3 months during the first year to achieve baseline (BL) classification as LV-AC or IC, thereafter every 3–6 months. HBeAg serum levels (Architect-QT) were tested at least once a year. HBsAg EOF line (BL) classification as LV-AC or IC, thereafter every 3–6 months. HBeAg serum levels (Architect-QT) were tested at least once a year. Factors influencing the end of follow-up (EOF) outcomes were analyzed by multivariate analysis (SSPS-20).

**Results:** BL characteristics and HBsAg levels are shown in the table.

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<table>
<thead>
<tr>
<th>Variables</th>
<th>Category</th>
<th>AGE (years)</th>
<th>Mean ± SD</th>
<th>HBV-DNA (log10 UI/mL)</th>
<th>Mean ± SD</th>
<th>ALT (U/L)</th>
<th>Mean ± SD</th>
<th>HBSAg BL (log10 UI/mL)</th>
<th>Mean ± SD</th>
<th>Follow-up (months)</th>
<th>Overall mean ± SD</th>
<th>HBSAg EOF (log10 UI/mL)</th>
<th>Mean ± SD</th>
<th>HBSAg log10 decline</th>
<th>Mean ± SD</th>
<th>HBSAg log10 decline/year</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC (87, 49M, 38F)</td>
<td>48.2 ± 13.4</td>
<td>1.91 ± 0.81</td>
<td>26.1 ± 18.2</td>
<td>2.22 ± 1.36</td>
<td>59.6 ± 39.7</td>
<td>1.59 ± 1.94</td>
<td>0.63 ± 0.97</td>
<td>0.15 ± 0.27</td>
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<tr>
<td>LV-AC (46, 22M, 24F)</td>
<td>43.1 ± 10.9</td>
<td>3.43 ± 0.39</td>
<td>23.9 ± 9.08</td>
<td>3.04 ± 0.78</td>
<td>69.0 ± 44.2</td>
<td>2.73 ± 1.18</td>
<td>0.31 ± 0.62</td>
<td>0.06 ± 0.13</td>
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</tr>
<tr>
<td>IC vs LV-AC P value*</td>
<td>0.027</td>
<td>&lt;0.001</td>
<td>0.445</td>
<td>&lt;0.001</td>
<td>0.212</td>
<td>&lt;0.001</td>
<td>0.047</td>
<td>0.04</td>
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</tbody>
</table>

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**Methods:** 92 haemodialysis HCV patients were retrospectively reviewed. Thirty-six patients were treated with PEG-IFN α-2a (135 μg/week) plus Ribavirin (200 mg/day) for 24 or 48 weeks in respect to genotype. The dose of ribavirin was tailored as we already shown. Fifty-six patients were considered not eligible for treatment mainly for the very high cardiovascular risk. The primary end point was SVR. Univariate logistic regression was used to explore the association between the different variables and sustained viral response (SVR).

**Results:** Of the 36 treated patients, 12 (33%) were female, the mean age was 54 years (54 ± 9 years) and 18 (50%) had genotype 1. IC remained stable (79.4%) or cleared HBsAg (12.8%) and only 1 showed transition to LV-AC (0.8%). LV-AC remained stable (45.7%) or became IC (47.8%) with HBsAg loss in a minority (4.3%). CHB reactivation occurred in 1 case only (2.2%). LV-AC to IC transition was associated with lower BL-HBsAg (P = 0.032). HBsAg loss in IC was associated with lower BL-HBsAg and log10 HBsAg yearly decline (P = 0.001 and P = 0.005).

**Conclusions:** Inactive and low viremic HBV carriers, have long-term benign clinical outcomes associated with significant rates of HBsAg loss. This study provides evidence on the expert opinion statement of the 2012 EASL Clinical Practice Guidelines, suggesting antiviral treatment only in patients with HBV-DNA higher than 20,000 IU/ml.

http://dx.doi.org/10.1016/j.jldl.2014.01.032
OC-28

FOUR YEARS OF TENOFUROIR MONOTHERAPY FOR NUC NAÏVE FIELD PRACTICE EUROPEAN PATIENTS SUPPRESSES HBV REPLICATION IN MOST PATIENTS WITH A FAVORABLE RENAL SAFETY PROFILE BUT DOES NOT PREVENT HCC IN PATIENTS WITH OR WITHOUT CIRRHOSIS


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While Tenofovir (TDF) has become a popular anti-HBV strategy for naïve patients worldwide, its long-term effectiveness and safety in field practice is unknown. Methods: 374 naïve CHB patients (55 years, 73% males, HBV-DNA 6.0 log units, 80% HBeAg-negative, 35% cirrhotics) were treated with TDF for 39 months (range 0–72) in a European multicenter study. Safety focused on glomerular and tubular renal function. Results. Virological responses increased over time reaching 97% at year 4, independently of HBeAg status. The 4-year probability of HBeAg seroconversion was 37%, 17% patients cleared HBsAg (11 HBeAg-positive patients), 6 successfully stopped TDF. Partial virological response rates progressively declined from 14% at month 12 [44 IU/ml (10–264,000)] to 3% at month 30 [27 IU/ml (12–1,040)]. Transient virological breakthroughs, but no resistance, were observed in few patients. Creatinine and phosphorus levels remained unchanged over time while eGFR declined from 84 to 80 ml/min. The proportion of patients with eGFR <50 and <60 ml/min (MDRD) increased to 3% and 11% at year 4 respectively while 1% had phosphate <2.0 mg throughout the study. TDF dose was adjusted in 19 (5%) patients for renal events (eGFR decline in 17; low phosphate in 2) and discontinued in additional 7 (2%) patients who were switched to ETV (Overall, renal events in 26 patients, 7%). Nine additional patients were switched to ETV because of nonrenal related side effects. HCC developed in 10 compensated cirrhotics (4-year probability: 17%) and in 6 noncirrhotics (4-year probability: 4%) while no patient decompensated. Overall, 3.7% of patients died (7 for HCC, 1 liver failure, 6 extrahepatic) and 1.6% underwent liver transplantation (4 with HCC, 2 with baseline compensated disease). In conclusion, 4 years of TDF suppressed HBV replication in most treatment-naïve field practice European patients with CHB without any major renal safety signal but this treatment failed to prevent HCC.

http://dx.doi.org/10.1016/j.dld.2014.01.033

OC-29

ENRICHMENT IN NOVEL RARE CODING VARIANTS OF TELOMERASE GENE IN HEPATOCELLULAR CARCINOMA PATIENTS WITH NAS PH RELATED CIRRHOSIS

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Background and aims: In an increasing proportion of cases, hepatocellular carcinoma (HCC) develops in nonalcoholic steatohepatitis (NASH)-related liver disease. Mutations in human Telomerase reverse-transcriptase (hTERT) involved in hepatocytes proliferation and liver regeneration are associated with a spectrum of progressive familial liver disease, characterized by steatosis, and represent a risk factor for cirrhosis. Genetic epidemiology estimates that rare functional variants in a specific protein occur in roughly 1% of individuals, and in >70% of cases are mildly/frankly deleteri-
HCV is the only virus infecting humans, able to induce two different involved in the pathogenesis of non-Hodgkin's lymphoma (NHL). of death for malignancy worldwide, and its association with HCV was limited to NHL patients. 

Patients and methods: In 14 consecutive unrelated patients with HCC in NASH-related disease or cirrhosis, or cryptogenic cirrhosis likely resulting from NASH, we re-sequenced hTERT/hTERC coding regions and intron–exon boundaries. 

Results: We detected novel rare coding variants of hTERT in three patients: Ala67Val in homozygosity, and Pro193Leu and Glu668Asp in heterozygosity (prevalence 21.5%, p = 0.013 vs. expected rare coding alleles frequency). No mutations were detected in hTERC. Of patients positive for TERT mutations, two were men and one female, mean age was 70 ± 8 years. All presented with a single HCC lesion (p = 0.05 vs. negative patients) of 3.5 ± 1.4 cm (vs. 3.8 ± 1.2) in previously undiagnosed liver disease with compensated cirrhosis (p = 0.06), all had diabetes and were positive for the I148M PNPLA3 variant predisposing to HCC. Mortality was 100% (vs. 65% in negative) at 24 months.

Conclusions: TERT mutations are enriched in patients with NASH–related HCC, may be associated with peculiar clinical features and a poor outcome, and may have implications for family screening in relatives.

http://dx.doi.org/10.1016/j.jdd.2014.01.034

OC-30 DYSREGULATION OF MICRORNA EXPRESSION IN PBMCs FROM PATIENTS WITH HCV-RELATED MALIGNANCIES A. Piluso 1, E. Fognani 1, L. Gragnani 1, E. Grandini 2, M. Monti 1, P. Caini 1, T. Urraro 1, B. Boldrini 1, M. Bernardi 2, G. Laffi 1, P. Andreone 2, A.L. Zignego 1

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Introduction: Hepatocellular carcinoma (HCC) is a major cause of death for malignancy worldwide, and its association with HCV infection has been definitively established. Besides HCC, HCV is involved in the pathogenesis of non-Hodgkin’s lymphoma (NHL). HCV is the only virus infecting humans, able to induce two different malignancies.

Aim: We previously showed a down-regulation of miR-26b in peripheral blood mononuclear cells (PBMCs) from patients with HCV-related mixed cryoglobulinemia (MC) or NHL, and an up-regulation of miR-16, miR-21 and miR-155 in NHL patients.

In this study, we analyzed the expression of the same panel of malignancy-associated microRNAs also in PBMCs from HCV patients with or without HCC.

The comparative analysis of miRNA expression between hepatic and lymphatic malignancies could provide some hints on the issue of a differential evolution of HCV infection to HCC or NHL, suggesting the existence of common or distinct pathogenetic pathways and identify new useful biomarkers.

Methods: The expression of miR-16, miR-21, miR-26b, miR-146a and miR-155 was analyzed by Real-Time PCR, using miR-let-7b as endogenous control.

Results: Data obtained showed the up-regulation of miR-21 and down-regulation of miR-26b in HCC patients compared to controls (p < 0.001). MiR-146a levels were comparable in patients and controls. The expression of miR-16 and miR-155 did not differ in HCC patients and controls, indicating that their deregulated expression was limited to NHL patients.

Conclusion: This study shows that some microRNAs are differently expressed in PBMCs from HCV patients who developed HCC or NHL, while others share a common behavior. Thus, the analysis of the expression of microRNAs could represent a non-invasive markers of HCV-related cancerogenesis; this could be suitable both to identify the existence of a malignancy and to discriminate between the two major HCV-related cancers.

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Background: The role of hepatic resection for patients with hepatocellular carcinoma (HCC) in different Barcelona Clinic Liver Cancer (BCLC) stages remains controversial due to the scarcity of randomized controlled trials and the intrinsic prognostic heterogeneity within each BCLC stage. Two large prospective databases were merged to measure the net survival benefit of hepatic resection over non-surgical-therapies in each BCLC stage.

Methods: Using BCLC stage D, extra-hepatic metastases, and liver transplantation as exclusion criteria, we selected 4713 consecutive HCC patients from one Eastern (n = 2266) and one Western (n = 2447) database. We performed three independent multivariate Cox survival analyses including patient-, liver function-, and tumor-related covariates within subgroups who underwent resection (n = 1340), loco-regional (n = 2406), or supportive care (n = 967). The obtained models were then used to predict individual 5-year life expectancy (LE) with resection compared to loco-regional therapy (LRT) or best supportive care (BSC), in each enrolled patient independently from therapy actually received. The results were expressed as net benefit of resection (proportion of LE variation due to resection over LRT weighted for BSC) using the formula: (LE-resection – LE-LRT)/LE-BSC.

Results: Multivariate Cox survival analysis included the following variables: patient-related (age, nationality), liver function-related (child class and portal hypertension), tumor-related (node size, number, and alpha-fetoprotein), and BCLC staging, while treatment (resection, LRT, or BSC) was included as stratifying covariate.

Mean net benefit (95% confidence interval) of resection over LRT significantly increased according to BCLC stage: BCLC 0 = 9.75% (6.04–13.47), A = 13.82% (11.99–15.66), B = 31.46% (28.93–33.98), C = 46.02% (44.39–47.65). Diameter of the largest nodule was the main contributor to this model.
Conclusion: Hepatic resection may result in net survival benefit for HCC patients regardless BCLC stage, provided that technical feasibility and oncological radicality are guaranteed.

http://dx.doi.org/10.1016/j.dld.2014.01.036

OC-32

A REGRET-BASED APPROACH TO CHOOSE BETWEEN TRANS-CATHETER ARTERIAL EMBOLIZATION AND HEPATIC RESECTION FOR INTERMEDIATE HEPATOCELLULAR CARCINOMA

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Introduction: Trans-catheter arterial chemo-embolization (TACE) is the first-line therapy recommended by western guidelines for intermediate hepatocellular carcinoma (HCC); however, in clinical practice, such patients are often referred to surgical teams for evaluation and treatment. After making a decision under uncertainty, physicians may discover that the alternative approach would have been preferable, imparting a sense of regret. Regret theory postulates that the optimal choice would be the one associated with the least amount of regret until the case it is proven wrong.

Aim: To apply regret theory to the decision-making of treatment of intermediate HCC.

Methods: Data from 247 cirrhotic patients, resected for intermediate HCC, were used to build a prognostic model and to compute a regret decision-curve analysis (DCA) integrating physician’s preferences expressed in terms of regret associated with surgery and TACE choices. Physician’s treatment preferences were indicated by a threshold probability (Pt) at which the physician is uncertain whether or not perform surgery. A survey among 40 hepatologists and surgeons regarding three hypothetical clinical cases was performed to assess if the physicians’ preferences cluster within relatively narrow domains.

Results: The 3- and 5-year overall survival rates after surgery were 48.7% and 33.8%, respectively. Child-Pugh score, tumor number and presence of oesophageal varices were independent predictors of overall survival after hepatectomy (P < 0.05). Regret DCA showed that the use of the prediction model was associated with the least amount of regret until Pt = 70%, above which TACE of all patients was the least regretful strategy. The survey pointed to, on average, a significant separation among physicians’ preferences, pointing to the need for separate elicitation of individual preferences of each decision-maker. However, the use of regret DCA uniformed final decisions.

Conclusions: Regret theory provides a new perspective for treatment-related decisions and can be applied in the setting of treatment of intermediate HCC.

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http://dx.doi.org/10.1016/j.dld.2014.01.036

OC-33

LOW ACCURACY OF NON-INVASIVE TESTS FOR ASSESSING RESIDUAL CIRRHOSIS IN HEPATITIS C PATIENTS WITH A SUSTAINED VIROLOGICAL RESPONSE

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Background: A sustained virological response (SVR) to anti-HCV therapy can result in fibrosis and cirrhosis regression. A recent study demonstrated that the accuracy of transient elastography (TE) in assessing cirrhosis regression after an SVR is low, while the performance of other non-invasive tests is still unclear.

Aim: To investigate the accuracy of non-invasive tests for assessing residual cirrhosis in HCV patients after an SVR.

Methods: All HCV patients with a pre-treatment histological diagnosis of cirrhosis and available post-SVR liver biopsies had residual liver fibrosis assessed through the following non-invasive methods: APRI, CDS, Fib4, FibroQ, Forns score, Guci Index, King score, Lok Index, PLF. Liver fibrosis staged according to the METAVIR score was the reference standard. The performances of non-invasive tests to diagnose residual cirrhosis were calculated using receiver operating characteristic (ROC) curves analysis.

Results: 20 out of 33 patients (61%) included in the study had cirrhosis regression after 61 (48–104) months from an SVR. The overall diagnostic accuracy of all the noninvasive serum panels analyzed was suboptimal as indicated by the AUROC values and the operative characteristics of the tests. None of these tests is useful in identifying patients with residual cirrhosis when using both the cut-off indicated by the literature (bold) and the cut-off with the best sensitivity and specificity derived from the ROC curves (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Test</th>
<th>Cut off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>LR+</th>
<th>LR-</th>
<th>AUROC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>APRI</td>
<td>&gt;0.2</td>
<td>84</td>
<td>30</td>
<td>1.2</td>
<td>0.5</td>
<td>0.58</td>
<td>(0.39-0.75)</td>
</tr>
<tr>
<td>CDS</td>
<td>&gt;5</td>
<td>46</td>
<td>75</td>
<td>1.8</td>
<td>0.6</td>
<td>0.60</td>
<td>(0.41-0.76)</td>
</tr>
<tr>
<td>FIB-4</td>
<td>&gt;3.25</td>
<td>15</td>
<td>99</td>
<td>15</td>
<td>0.8</td>
<td>0.59</td>
<td>(0.41-0.76)</td>
</tr>
<tr>
<td>Fibro Q</td>
<td>&gt;2.6</td>
<td>85</td>
<td>25</td>
<td>1.1</td>
<td>0.6</td>
<td>0.61</td>
<td>(0.42-0.78)</td>
</tr>
<tr>
<td>Forns score</td>
<td>&gt;6.9</td>
<td>23</td>
<td>90</td>
<td>2.3</td>
<td>0.8</td>
<td>0.52</td>
<td>(0.34-0.70)</td>
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<tr>
<td>Guci index</td>
<td>&gt;0.26</td>
<td>76</td>
<td>30</td>
<td>1.1</td>
<td>0.7</td>
<td>0.57</td>
<td>(0.39-0.74)</td>
</tr>
<tr>
<td>King score</td>
<td>&gt;16.7</td>
<td>15</td>
<td>99</td>
<td>15</td>
<td>0.8</td>
<td>0.59</td>
<td>(0.40-0.75)</td>
</tr>
<tr>
<td>Lok index</td>
<td>&gt;10.1</td>
<td>46</td>
<td>85</td>
<td>3.1</td>
<td>0.6</td>
<td>0.57</td>
<td>(0.39-0.74)</td>
</tr>
<tr>
<td>PLF</td>
<td>&gt;2.98</td>
<td>31</td>
<td>95</td>
<td>6.5</td>
<td>0.7</td>
<td>0.75</td>
<td>(0.57-0.89)</td>
</tr>
</tbody>
</table>

All the patients had values lower than the standard cut off.

Conclusions: Most of non-invasive serologic tests are not accurate in assessing fibrosis stage and identifying cirrhotos after an SVR. Histological evaluation through liver biopsy still remains the gold standard in these patients.

http://dx.doi.org/10.1016/j.dld.2014.01.038
OC-34

APPEARANCE OF COMBINED HEPATOCELLULAR CHOLANGIOCARCINOMA IN CIRRHOSIS AT CONTRAST ENHANCED IMAGING TECHNIQUES

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Background and aim: Combined hepatocellular-cholangiocarcinoma (CHC) is a rare primary liver cancer being reported in cirrhotic patients. Non-invasive differentiation between hepatocellular carcinoma (HCC) and other malignant nodules found in cirrhosis is critical. Contrast-enhanced ultrasound (CEUS) has been excluded from EASL/AASLD guidelines due to its difficulty in distinguishing HCC from intrahepatic cholangiocarcinoma. Scant data exist about contrast-enhancement appearance of CHC on cirrhosis. Aim was to evaluate the enhancement pattern in the vascular phases of CHC on cirrhosis at CEUS, CT or MRI. Secondary aim was the rate of CHC at risk of misdiagnosis for HCC.

Methods: All histologically confirmed CHC on cirrhosis seen in two Italian centers (Bologna, Milan) between 2003 and 2013 in which at least one imaging technique (CEUS, TC or MRI) had been performed, were retrospectively collected. The enhancement pattern was analyzed at all available imaging modalities.

Results: A total of 38 CHC nodules were identified. CEUS, CT and MRI were performed in 27, 35 and 17 nodules, respectively. No specific contrast pattern was observed in CHC nodules, although rim-like arterial enhancement (atypical for HCC) was found in 27%, 56% and 23% nodules at CEUS, CT and MRI, respectively. CEUS was at risk of misdiagnosis of CHC for pure HCC (hallmark = arterial homogeneous enhancement followed by wash out) in a higher number of cases than CT (48% vs. 23%, \( p = 0.024 \)) or MRI (48% vs. 33%, \( p = \) ns). Only 6 of 21 CHC lesion submitted to both CEUS and CT showed coincident enhancement patterns; CEUS suggested a condition of malignancy (presence of venous wash-out after any type of arterial hyperenhancement) in a higher number of cases than CT (71% and 29% respectively, \( p = 0.013 \)).

Conclusion: CEUS misdiagnosed as HCC a higher number of CHC on cirrhosis than CT and MRI, however the latter two techniques less often were able to identify signs of malignancy.

http://dx.doi.org/10.1016/j.dld.2014.01.039
THURSDAY

T-01

MANAGEMENT OF INFECTIOUS MIXED CRYOGLOBULINEMIA: CLINICAL OUTCOME OF 246 PATIENTS

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Introduction: The clinical and therapeutic management of infectious mixed cryoglobulinemia (MC) is not well known.

Material and methods: We enrolled 246 patients with MC hepatitis C and hepatitis B-related consecutively admitted to our Departments from 1995 to 2012, followed-up until march 2013 (median 9.2 years).

Results: At diagnosis median age was 60 years (range 26–83), 65% female. Etiology was HCV in 95%, HBV in 3% and “essential” in 2%. HCV genotype 1b was in 57%, genotype 2–3 in 43%. Mixed cryoglobulinemia was type II in 203 (87%) cases and type III in 52 (13%). More frequent clinical manifestations were purpura (72%), arthralgias (58%), peripheral neuropathy (21%), cutaneous ulcers (3%), chronic liver disease (70%), glomerulonephritis (35%), and non Hodgkin lymphoma (15%). Purpura, arthralgia, peripheral neuropathy, and non Hodgkin lymphoma were more frequently observed in type II, than in type III patients (p < 0.05). Treatment was interferon alone or plus ribavirin in 101 cases, steroids alone or plus alkylating agents in 33 cases, rituximab in 8 cases. More frequently complete clinical, virological and immunological responses were associated to interferon plus ribavirin. Severe infections were associated to steroids high dose and to alkylating agents. Finally, at 10 years, the survival in type II cryoglobulinemia was 71% and in type III 84% (p = 0.053).

Conclusions: Interferon plus ribavirin should be considered the first line therapy in MC HCV related, whereas steroids, alkylating agents and rituximab as second-line therapy. The role of these therapeutic strategies should be proved in randomized controlled trials.

http://dx.doi.org/10.1016/j.dld.2014.01.041

T-02

HEPATITIS C VIRUS IN HEALTHY IMMIGRANTS: TO SCREEN OR NOT TO SCREEN?

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Introduction: Hepatitis viruses screening is a secondary prevention strategy to detect earlier liver diseases and begin precociously antiviral treatment to prevent liver disease progression. Worldwide HCV prevalence is generally low and only in few countries is higher than 3.5%. Immigrants in Italy come predominantly from Eastern Europe, Asia and Africa. In all these areas HCV prevalence is lower than HBV.

Aim: The aim of this study was to assess the prevalence of HCV in the same population of healthy immigrants residing in Padua tested for HBV.

Materials and methods: Regular healthy immigrants were sent to our clinic by community leaders from March 2013 to October 2013, questioned about their sociodemographic characteristics, tested for HBV and also for HCV-ab. HCV-ab +ve subjects were tested also for HCV-RNA and HCV genotype.

Results: 450 (264 – M 58.7% and 185 – F 41.3%) immigrants were screened. 39% were from Eastern Europe, 23% from Asia, 36% from Africa, and 2% from other areas. This distribution is comparable
with immigrants residing in Padua. 31 (7%) were HBsAg +ve, 8 (1.9%) were HCV-ab +ve. Only 4 (50%) resulted HCV-RNA +ve and all were genotype 1b. Interestingly all the HCV-positive subjects belong to the Eastern European group with a prevalence of 8/174 (4.6%), while in the Asian group (104 subjects) and in African group (185 subjects) there were no HCV-positive subjects. All the HCV +ve subjects were over 45 yr.

Conclusions: The prevalence of HCV in Padua immigrants seems to be very low unlike HBV. HCV screening for immigrants does not appear useful to identify Hepatitis C virus affected subjects. HCV screening strategy could be effective only in special populations of immigrants with higher HCV prevalence (i.e. East Europe).

http://dx.doi.org/10.1016/j.jdd.2014.01.042

T-03

PEG-INTERFERON AND RIBAVIRIN THERAPY IS SAFE AND EFFECTIVE AMONG HCV PATIENTS WITH THALASSAEMIA MAJOR

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Background: Chronic hepatitis C (CHC) is a major cause of liver-related mortality in patients with thalassaemia major (TM). Peg-interferon (PegIFN) plus ribavirin (RBV) combination (PR) is the standard of care but little is known about iron profile modifications induced by anti-HCV therapy.

Aim: To assess efficacy and safety of PR in TM-patients and to evaluate the influence of PR on haematological parameters.

Materials and methods: TM-patients with CHC consecutively treated with PR in 3 Italian Centers were evaluated

Results: Seventy-four TM-patients (47% males, 86% treatment-naive, HCV-1 51%, HCV-2 39%, HCV-3 3% and HCV-4 7%) received treatment. 49 (66%) achieved a SVR (66% HCV-1, 69% HCV-2, 50% HCV-3 and 60% HCV-4). RBV was reduced in 22% patients after 4–66 weeks, and PegIFN in 22% after 2–36 weeks, without any impact on SVR rates ($p<0.07$ and 0.77, respectively). PR was withdrawn in 5% due to anaemia-unrelated AEs. Ferritin values, blood requirement and iron intake increased on-treatment and decreased during follow-up (Baseline vs EOT vs 6-month follow-up: 684 vs 1440 vs 710 ng/ml; 0.51 vs 0.75 vs 0.55 mg/kg/die; 0.34 vs 0.56 vs 0.39 mg/kg/die; $p<0.001$ for all comparisons) with no differences between SVR and non-SVR patients.

Conclusions: PR efficacy and safety profiles in TM patients are similar to those reported in the general population. Anti-HCV treatment results in iron overload due to the increased need for blood transfusion, however it is reversible after EOT.

http://dx.doi.org/10.1016/j.jdd.2014.01.043

T-04

PNPLA3 RS738409 C > G SNP IS ASSOCIATED WITH HISTOLOGICAL FEATURES OF NASH IN GENOTYPE 1 CHRONIC HEPATITIS C PATIENTS

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Background/Aims: PNPLA3 rs738409 C > G SNP is associated in subjects with NAFLD to a diagnosis of NASH, and in patients with CHC with the severity of steatosis and fibrosis. We assessed in patients with GT1 CHC the relation between PNPLA3 SNPs and histological features of NASH, and of PNPLA3 SNP and NASH on the severity of fibrosis.

Methods: 254 consecutive patients with GT1 CHC were studied. PNPLA3 rs738409 C > G SNP was assessed. Biopsies were scored for staging and grading (Scheuer). Steatosis and features of NASH were also assessed (Bedossa).

Results: Histological features of NASH were present in 31.5% of patients. PNPLA3 rs738409 C > G (“G” allele) was found in 40.9% (30.3% GT, 10.6% GG). A “G” allele was independently linked with steatosis $\geq$30% (OR 2.005, 95% CI 1.015–3.961, $p=0.04$) and its prevalence was higher in patients with features of NASH (40/80 in PNPLA3 “GG” carriers vs 64/174 in PNPLA3 “CC”, $p=0.04$), this association being confirmed at multivariate analysis (OR 2.109, 95% CI 1.194–3.726, $p=0.001$) after correction for age and HOMA. Patients with $\geq$F3 fibrosis had a comparable prevalence of PNPLA3 alleles (29/74 in “GG” carriers vs 75/180 in CC, $p=0.71$), with a higher prevalence of NASH compared to no NASH (39/74 vs 41/180, p = 0.001). This was confirmed after correction for age, severe necroinflammatory activity, and steatosis $\geq$30% (OR 2.466, 95% CI 1.271–4.782, p = 0.008).

Conclusions: The PNPLA3 G > C SNP has a major link to histological features of NASH among GT1 CHC patients, thus being indirectly to the severity of fibrosis.

http://dx.doi.org/10.1016/j.jdd.2014.01.044

T-05

PEG-INTERFERON PLUS RIBAVIRIN WITH OR WITHOUT BOCEPREVIR OR TELAPREVIR FOR HCV GENOTYPE 1: A META-ANALYSIS ON ROLE OF RESPONSE PREDICTORS

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Aims: To compare the efficacy of pegylated-interferon (Peg-IFN) α-2a or α-2b and ribavirin given as dual therapy vs. triple therapy (Peg-IFN and ribavirin plus boceprevir or telaprevir) in patients with HCV-1 chronic hepatitis naïve to anti-HCV therapy or relapsers to dual therapy to identify most adequate treatment
strategies for patients' subgroups with high rate of sustained viral response (SVR).

Methods: Included in the meta-analysis were studies meting these criteria: original data from randomized trials on the efficacy of dual versus triple therapy in patients naive or relapse; at least one primary outcome clearly defined: SVR with or without rapid virological response (RVR), with genotype 1a or 1b, low or high HCV viral load, IL28-B CC or non-CC, mild or severe fibrosis; odds ratio estimates of relative risk (RR) and 95% confidence intervals (CIs); English language; published within June 2013.

Results: Seven original studies met inclusion criteria, allowing a meta-analysis on 3652 patients. Triple therapy was more effective than dual, regardless of IL-28B genotype, HCV sub-genotype, liver fibrosis, and baseline HCV load. In 1045 patients who achieved RVR, SVR was more frequently obtained with dual therapy (RR = 1.11; p = 0.002) than triple. In naïve patients with low baseline HCV load, dual and triple therapy obtained similar SVR rates (RR = 0.93).

Conclusion: Triple therapy provides a significantly higher SVR rate than dual therapy, but dual therapy obtains a significantly higher SVR rate in patients with RVR. The data stress the clinical relevance of a 4 weeks lead-in phase in DAA-based treatment.

http://dx.doi.org/10.1016/j.dld.2014.01.045

T-06
THE HISTOLOGICAL OR ULTRASONOGRAPHIC DETECTION OF STEATOsis AFFECTS THE PERFORMANCE OF LSM IN PATIENTS WITH CHRONIC HCV GENOTYPE 1 INFECTION
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2 Cattedra di Anatomia Patologica, University of Palermo, Palermo, Italy

Background and aim: In chronic hepatitis C (CHC), the influence of steatosis on liver stiffness measurement (LSM) is still debated. We assessed the impact of steatosis and its ultrasonographical sign – i.e. bright liver echo pattern (BLEP) – on LSM values and on transient elastography (TE) accuracy for the diagnosis of liver fibrosis, in a cohort of consecutive biopsy-proven patients with genotype 1 (G1) CHC.

Patients and methods: Consecutive G1 CHC patients (n = 618), assessed by liver biopsy (Scheuer score), anthropometric, biochemical, ultrasonographic and metabolical features, were included. TE was assessed using the standard M probe. Steatosis was considered moderate–severe if ≥20%.

Results: Male gender (p = 0.04), steatosis as continuous variable (p < 0.001), severity of necroinflammation (p = 0.02) and stage of fibrosis (p < 0.001) were associated with LSM by multivariate linear regression analysis. Sensitivity, specificity, positive predictive value and negative predictive value of ultrasonography (US) for the diagnosis of steatosis ≥20% were 67.7%, 78.2%, 51.0% and 86.4%, respectively. Among patients within the same fibrosis stages (F0–F2 and F3–F4; F0–F3 and F4), mean LSM values, expressed in kPa, were significantly higher in subjects with moderate–severe steatosis (≥20%) compared with those without, as well as in patients with BLEP on US compared with those without. Furthermore, in subjects without severe fibrosis (F0–F2) and without cirrhosis (F0–F3), a higher rate of false-positive LSM results was observed in patients with steatosis ≥20% (F0–F2: 35.3% vs. 17.9%; F0–F3: 38.9% vs. 16.6%)

and in patients with BLEP on US (F0–F2: 28.0% vs. 18.3%; F0–F3: 29.7% vs. 17.8%) compared with their counterparts.

Conclusions: In patients with G1 CHC, the presence of moderate–severe steatosis, detected by histology or by US, should always be taken into account in order to avoid overestimations of liver fibrosis assessed by LSM.

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T-07
INOSINE TRIPHOSPHATASE DEFICIENCY DOES NOT PREDICT ANEMIA SEVERITY NOR ANEMIA MANAGEMENT IN PATIENTS WITH ADVANCED FIBROSIS RECEIVING TELAPREVIR
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Background: Anemia during the first 12 week of therapy is the most common side effect of pegylated interferon (PegIFN)/ribavirin (RBV) and telaprevir (TVR) in HCV genotype 1 patients with advanced fibrosis or cirrhosis (F3–F4). Inosine triphosphatase (ITPA) genetic variants are associated with RBV-induced anemia and dose reduction but their role as predictors of anemia during TVR therapy is unknown.

Aim: To test the association of ITPA polymorphisms rs1127354 and rs7270101 with hemoglobin (Hb) decline, need for RBV dose reduction (RBV DR), erythropoietin (EPO) support and blood transfusions during the first 12 weeks of triple therapy.

Materials and methods: 69 consecutive HCV-1 patients with F3–F4 treated with PegIFNα/ RBV and TVR 750 mg/Q8 h were genotyped for ITPA polymorphisms rs1127354 and rs7270101 using TaqMan probes. Estimated ITPA deficiency was graded on severity (0–3, no deficiency/mild/moderate/severe).

Results: Patients mean age was 57 years, 57 (83%) had HCV-1b, 9 (13%) were treatment naive and 51 (74%) had cirrhosis. No ITPA deficiency was found in 48 patients (70%), mild deficiency was found in 12 (17%) and moderate in 9 patients (13%). Mean week 4 Hb decline was higher in non-ITPA deficient patients (3.85 g/dL) than in mild ITPA deficient patients (3.07 g/dL) and moderate deficient patients (1.67 g/dL) (p < 0.0001). Grade of ITPA deficiency was not associated with RBV DR (no deficiency: 60%, mild deficiency: 58%, moderate deficiency: 67%; p = ns), EPO use (no deficiency: 65%, mild deficiency: 58%, moderate deficiency: 56%; p = ns) or need for blood transfusion (no deficiency: 27%, mild deficiency: 17%, moderate deficiency: 33%; p = ns). Grade 3–4 anemia developed in 81% of non-ITPA deficient patients versus 67% of mild deficient patients and 56% of moderate deficient patients (p = ns).

Conclusions: In patients with F3–F4 chronic hepatitis C receiving TVR based therapy, ITPA genotype does not impact on the management of early anemia.

http://dx.doi.org/10.1016/j.dld.2014.01.047
T-08

LINKAGE BETWEEN INTERFERON-L4 AND IL28B POLYMORPHISMS IN HCV-4 PATIENTS TREATED WITH PEGYLATED INTERFERON AND RIBAVIRIN

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Aim: In this study, we compared the role of IFNL4 polymorphism with the commonly used IL28B rs12979860 on response to Peg-interferon (PEG) and ribavirin (Ribv) therapy.

Materials and methods: 80 naïve to antiviral treatment patients, 72 (90%) males, 54 (67%) of Egyptian origins, 14 (18%) cirrhotics, had the IFNL4 gene sequenced by Sanger method and IL28B patients, 72 (90%) males, 54 (67%) of Egyptian origins, 14 (18%) cirrhotics, had the IFNL4 gene sequenced by Sanger method and IL28B

Results: Genotype distributions of the IFNL4 ss469415590 TT > ΔG, which is in high linkage disequilibrium (LD) with IL28B rs12979860 C > T polymorphism. The ss469415590 (ΔG) allele encodes the novel IFNL4 protein which could be associated with impaired response to Peg-interferon (PEG) and ribavirin (Ribv) therapy.

Conclusions: Our data confirm the strong LD between ss469415590 and rs12979860 variants showing that in HCV-4 patients IFNL4 genotyping provides no additional information for treatment prediction compared to IL28B.

http://dx.doi.org/10.1016/j.dld.2014.01.048

T-09

CANNABINOIDS RECEPTOR 2-63 QQ VARIANT IS ASSOCIATED WITH PERSISTENTLY NORMAL AMINOTRANSFERASE SERUM LEVELS IN CHRONIC HEPATITIS C

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Aims: To evaluate in anti-HCV-positive patients the clinical impact of the rs35761398 single nucleotide polymorphism (SNP) of the CNR2 gene leading to the substitution of Arg (R) of codon 63 of the cannabinoid receptor 2 (CB2) with Cln (Q).

Patients and methods: 253 consecutive anti-HCV/HCV-RNA-positive patients were enrolled: males 53%; median age 52 years (range 18–80), mean serum HCV-RNA 2.6 × 10E6 IU/ml (SD: ±5.6 × 10E6), 63.6% with genotype 1, 73.5% with liver biopsy (Histological Activity Index: 5.93 ± 3.5; fibrosis score according Ishak score 2.3 ± 1.42; steatosis score: 1.19 ± 1.2). Of these 253 patients, 53 were HCV carriers with persistently normal ALT (PNALT group) and 200 had a history of steadily abnormal serum ALT values (abnormal ALT group). All patients were naïve for antiviral therapy and were screened for CNR2 rs35761398 SNP by a real-time assay.

Results: Subjects in the PNALT group, compared with those in the abnormal ALT group were older (58.5 ± 12 vs. 50.7 ± 12.4 years, p = 0.001), more frequently female (66% vs. 42%, p = 0.003), with lower body max index (24.5 ± 3.1 vs. 26.6 ± 4.6, p = 0.003), and more frequently with HCV genotype 2 (43.1% vs. 17.7%, p = 0.0002) and CB2–63 QQ variant (34% vs. 11%, p = 0.0001).

Considering all 253 patients, no difference in the demographic, biochemical, or virological data was observed between patients in the different CB2–63 variants. The logistic regression analysis identified CB2–63 QQ, HCV genotype 2, older age and lower BMI as independent predictors of PNALT (p < 0.00001).

Discussion: The CB2–63 QQ variant in HCV patients was independently associated to the PNALT status.

http://dx.doi.org/10.1016/j.dld.2014.01.049
T-10
MISSENSE VARIANT IN INTERFERON-Λ4 GENE IDENTIFIES HCV-1 INFECTED PATIENTS CARRYING THE UNFAVORABLE IL28B (T) ALLELE WITH IMPROVED VIRAL KINETICS
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Introduction: The interferon-λ4 gene (IFNL4) harbors the dinucleotide variant ss469415590 TT > ΔG in high linkage disequilibrium (LD) with interleukin 28B (IL28B) rs12979860 C > T polymorphism. The TT allele leads to a frameshift that inactivates the gene, while the ΔG allele results in translation of IFNL4 protein that could be the causal agent for the poor prognosis of hepatitis C virus genotype 1 (HCV-1) patients. However, three nonsynonymous variants found within IFNL4 gene could modulate the IFNL4 function.

Aim: To elucidate if missense variants acts as modifiers of virological response, we performed direct sequences by Sanger method of IFNL4 gene in a well-characterized Italian cohort of 103 naïve HCV-1 patients treated with pegylated interferon-α (PEG) and ribavirin (RBV) for 48 weeks.

Results: We found a strong LD between ss469415590 and rs12979860 variants (r² = 0.98) showing that genotyping of the IFNL4 variant supplies information comparable to that of the IL28B. Since the rs117648444 (p.Pro70Ser) was the only missense variant identified in our samples, we stratified the 74 carriers of unfavorable IFNL4 (ΔG) allele into wild-type (WT: n = 53) and mutated (MUT: n = 21) patients. By analyzing on treatment HCV kinetics values, MUT patients had a more pronounced mean of HCV RNA decline at week 4 of therapy compared to WT (2.2 log 10 IU/mL vs 1.69 log 10 IU/mL, p = 0.02), which translated in a significant higher rate of rapid virological response (RVR), i.e. HCV RNA negativity at week 4 (p = 0.02). Importantly, these differences were not significant between IL28B CT and TT genotypes.

Conclusion: Stratifying the IFNL4 (ΔG)/IL28B (T) carriers according to ss117648444 missense variant we identified patients with a more pronounced HCV RNA decline at week 4 of PEG/RBV therapy, providing additional information to IL28B genotyping.

http://dx.doi.org/10.1016/j.dld.2014.01.050

T-11
LYMPHOCYTES AS LIVER DAMAGE MIRROR OF HCV RELATED ADIPOGENESIS Deregulation
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Hepatitis C virus infection leads to a wide spectrum of liver diseases ranging from mild chronic hepatitis to end-stage cirrhosis and hepatocellular carcinoma. An intriguing aspect of the HCV infection is its close connection with lipid metabolism playing an important role in the HCV life cycle and in its pathogenesis. HCV is known to be a hepatotropic virus; however, it can also infect peripheral blood mononuclear cells (PBMCs).

The goal of the current investigation is to compare the adipogenesis profile of liver tissues to lymphocytes of HCV infected patients, in order to understand if PBMCs may reflect the alterations of intracellular pathways occurring during HCV-related liver steatosis.

Using the Human Adipogenesis PCR Array, gene expression was analyzed in liver samples and PBMCs of chronic HCV+, HBV+ and Healthy Donors (HDs) patients.

We observed a similar modulation of lipid metabolism in HCV+ liver tissues and lymphoid cells, suggesting that PBMCs reflect the liver adipogenesis deregulation related to HCV infection. In particular, some genes involved in lipid metabolism and inflammation, as well as in cell transformation, were up-regulated, in a similar way, in both HCV models analyzed. Interestingly, these genes were positively correlated to virological and hepatic functional parameters of HCV+ patients. On the contrary, HBV+ patients displayed a completely different profile.

PBMCs of HCV+ patients seem to be useful model to study how HCV-related lipid metabolism deregulation occurs in liver.

The obtained data suggest some molecules as new possible biomarkers of HCV-related liver damage progression.

http://dx.doi.org/10.1016/j.dld.2014.01.051
22.9%. Treatment strategies used were: (a) TDF, (b) ETV, (c) LAM (add-on NUCs), (d) ADV (add-on strategy), (e) PEG IFN followed by TDF/ETV. Overall response rate in term of HBV-DNA negativity was 98%. Results of cost-effectiveness analysis are shown in table:

<table>
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<tr>
<th>Treatment</th>
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<th>ICER QALYs</th>
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<td>€ 2075</td>
<td>€ 2075</td>
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</tbody>
</table>

Conclusions: These results showed that the antiviral treatment for HBV with last generation NUCs in real-life setting is effective in over 98% of the patients. The most cost-effective strategy appears to be starting the treatment with TDF when compared with other anti-HBV treatments.

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T-13

LOW RISK OF HEPATITIS B VIRUS REACTIVATION IN HBsAG NEGATIVE, ANTI-HBc POSITIVE CARRIERS UNDERGOING RITUXIMAB FOR RHEUMATOID ARTHRITIS


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Background and aim: Safety of rituximab (RTX) in hepatitis B surface antigen (HBsAg) negative/anti-HBc positive patients with rheumatoid arthritis (RA) is unknown.

Patients and methods: We retrospectively reviewed 306 RA patients treated with RTX in 5 Italian outpatient rheumatologic Clinics. Complete serological screening for HBV status before RTX was available in 33 HBsAg negative/anti-HBc positive patients who did not undergo antiviral prophylaxis. These patients (73% female, 60 yrs, disease duration 8 yrs, 100% serum HBV DNA negative by PCR assay, 95% anti-HBs positive) have been treated with RTX plus disease-modifying anti-rheumatic drugs (DMARS). RTX was administered for 3 cycles (range: 1–8) lasting for 22 months (range: 0–62) in 14 patients and ongoing in the remaining cases. Laboratory examinations, including serum HBsAg and serum HBV DNA were assessed every 6 months and whenever ALT flared above the upper limit of normal.

Result: During 45 months (range: 12–80) of follow-up, anti-HBs titers dropped in 27% (2 patients became seronegative). All but one patient (3%) who showed a slight elevation in serum HBV DNA (44 IU/mL) maintained undetectable HBV DNA. In the latter patient, HBV DNA became detectable 5 months after the first RTX administration, was not associated to either HBsAg seroconversion or ALT flare and was promptly suppressed by lamivudine treatment. Another patient (3%) had an ALT flare that however was not related to HBV reactivation.

Conclusion: In HBsAg negative/anti-HBc positive RA patients, administration of RTX + DMARS poses a negligible risk of HBV reactivation thereby calling for HBsAg or HBV DNA monitoring, only.

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T-14

A COST-CONSEQUENCE ANALYSIS OF SCREENING AND TREATMENT FOR CHRONIC HEPATITIS B (CHB) VIRUS INFECTION IN RESIDENT IMMIGRANTS OF AN ITALIAN NORTH-EAST

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Introduction: The epidemiology of hepatitis B in Europe is changing, with migration causing significant increases in prevalence rates. It is of paramount importance to identify the most effective ways to contain the disease. Systematic screening and treatment of migrants for CHB virus infection is likely to be cost-effective, but it is crucial to take into account the significant associated costs and the considerable net investment by governments.

Aim: The objective of this study is to estimate the health and economic effects of screening strategy for CHB screening among immigrants.

Materials and methods: We used the Markov model to examine the cost-consequence of screening and treatment vs no screening strategy in a cohort of 348,991 adult migrants resident in the Veneto Region. The rate of adherence to the HBV screening program was judged to be 40%. The prevalence of HBV infection (6.03%) and the chance of having active CHB (30%) were based on our recent screening campaign in Padua involving 465 migrants. Likelihood of HBV-related events was obtained from literature.

Results: The screening-treatment strategy prevented 273 cases of cirrhosis, 18 decompensated cirrhosis, 28 HCC, and 54 CHB related deaths, over a period of 5 years. The incremental cost of the screening strategy totaled 51,597,980 € in five years (0.1% of the Veneto annual health budget).

Conclusions: This study provides information useful mainly to policy makers, who need to establish whether the cost generated by a screening strategy is affordable when set against the better health outcomes for resident immigrants.

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Background and aim: Aim of the study was to assess the efficacy and safety of entecavir (ETV) in LMV-ADV experienced patients who had to stop tenofovir (TDF) for tubular dysfunction.

Methods: 18 chronic hepatitis B patients (63 years, 94% males, 56% cirrhotics, 94% HBeAg-negative, 94% previously exposed to ADV and LMV, all with normal ALT and undetectable HBV DNA) who have been treated with TDF monotherapy for 39 (range 7–52) months, were switched to ETV monotherapy (dosing according to kidney function parameters, including TmPO4/GFR levels, were assessed at baseline (start ETV) and every 3 months.

Results: During 14 (range: 5–19) months of ETV monotherapy (1.0 mg/die in 8 patients), all renal parameters significantly improved: serum creatinine from 1.20 to 1.13 mg/dL, eGFR from 60 to 68 mL/min, serum phosphate from 2.2 to 2.4 mg/dL, and TmPO4/GFR levels from 0.42 to 0.52 mmol/L. While 9 (50%) improved hyperphosphaturia, none reached a normal TmPO4/GFR level. A virological breakthrough occurred in 5 (28%) patients between month 3 and 15 of ETV treatment. While for 2 patients the virological rebound was mild and transient (HBV DNA <40 IU/mL), TDF was rapidly reintroduced in the other 3 cases who developed ETV (2 cases) and LMV resistance (1 case).

Conclusions: TDF treated patients developing chronic tubular kidney dysfunction can be successfully rescued by ETV monotherapy with low risk of drug resistance.

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HBX DIRECTLY MEDIATES DEREGRATION OF SEVERAL NCRNAS IDENTIFIED BY CHIP-SEQ EXPERIMENT

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Introduction: HBx regulatory protein is required for HBV cccDNA transcription/viral replication and contributes to HBV oncogenicity. HBx affects the epigenetic control of HBV viral chromatin as well as of cellular chromatin. We have recently shown, using a genome wide chromatin immunoprecipitation approach (ChIP-Seq), that ~7000 genes and several miRNAs are potential direct targets of HBx.

Aim: Aim of this study was to extend our analysis of ncRNAs (micro and long non coding RNAs) targeted by HBx in HBV replicating cells.

Materials and methods: ChIP-Seq datasets were generated in HBV-replicating HepG2 cells. Chromatin immunoprecipitated from mock, wt and HBx-mt cells was analysed by TaqMan real-time PCR using miRNA and lncRNA promoter specific primers. HBx-targeted miRNAs and lnc-RNAs levels were assessed by Nanostring and RT-PCR.

Results: HBx binds to 232 miRNAs [99 putative miRNA promoters and 133 mirtrons], and to 39 long non coding RNAs. Functional analysis show that: (a) HBx can both upregulate and repress the expression of miRNAs that affect HBV replication (i.e. miR138, miR596 and others) and the control of cellular functions (i.e. miR21, miR26b); (b) HBx binding to miRNAs and lnc-RNA regulatory regions is accompanied by changes of chromatin modifying enzymes binding and histones epigenetic marks; HBx deregulates two long non coding RNAs (DLEU2 and SNHG12) leading to an aberrant transcription of the neighboring genes TRIM13 and BCLAF1, both of them implicated in apoptosis.

Conclusion: HBx is recruited to several genomic loci to modulate the epigenetic control of ncRNAs transcription.

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EPIGENETIC DRUGS TARGETING CCCDNA-BOUND CHROMATIN MODIFYING ENZYMES SILENCE HBV TRANSCRIPTION AND INHIBIT VIRAL REPLICATION

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Background and aims: The HBV cccDNA is organized into mini-chromosomes by histone and non-histone proteins and HBV replication is regulated by the acetylation status of cccDNA-bound H3/H4 histones. We have previously shown that: (a) interferon-α inhibits HBV transcription/replication by favoring the recruitment to the minichromosome of class III Histone Deacetylase hSirt1 and histone methyltransferase EzH2; (b) small compounds modulating these enzymatic activities may in part mimic IFNα-induced cccDNA silencing. Here we further characterize the activity on cccDNA transcription of small compounds active on different classes of chromatin modifying enzymes.

Methods: Capsid-associated HBV-DNA, cccDNA and pgRNA levels were assessed in HepG2 replicating HBV or in the inducible HepAD38 stable HBV cell line, left untreated or treated with: a) a p300 and PCAF histone acetyltransferases (HAT) inhibitor and (b) a hSirt1 activator. Recruitment of transcriptional cofactors and cccDNA bound histones modifications were assessed using the cccDNA ChIP assay.

Results: The inhibition of PCAF/p300 HATs or stimulation of hSirt1 activity resulted in an evident reduction of HBV replication that mirrored the decrease of pgRNA transcription. The HATs inhibitor induced a reduction of acetylation of the H4 bound to cccDNA and an inhibition of PCAF/p300 binding onto minichromosome, suggesting an autoregulatory loop involving p300 and PCAF acetylation and their binding to the cccDNA. The hSirt1 activator also induced a decrease in cccDNA bound H4 acetylation levels interfered without interfering with the Sirt1 recruitment onto cccDNA.

Conclusions: These results support the concept of an epigenetic approach with small molecules to modulate HBV transcription.

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EARLY MENOPAUSAL STATUS IS ASSOCIATED WITH THE SEVERITY OF LIVER FIBROSIS IN ITALIAN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and aims: Contrasting data have been reported on the effect of gender and menopausal status on the susceptibility to nonalcoholic fatty liver disease (NAFLD) development and liver damage progression in NAFLD. We assessed whether menopausal status is associated with severity of liver fibrosis in NAFLD.

Methods: In 244 consecutive females and 244 age-matched males with biopsy-proven NAFLD, from Italian referral centers, we assessed anthropometric, biochemical, and metabolic features, including menopausal status (self-reported); liver biopsy was scored according to Kleiner.
**Results:** In the entire cohort, at multivariate logistic regression adjusted for age, BMI, type 2 diabetes, serum lipids, and presence of NASH, male gender (OR 1.408, CI 0.779-2.542, p = 0.25) respect to women at reproductive age was not associated with fibrosis > F1, while a trend was observed for menopause (OR 1.752, CI 0.956-3.208, p = 0.06). Accordingly, in female patients menopause (OR 3.079, 95% CI 1.136-8.345, p = 0.02) was associated with fibrosis > F1, independently of the aforementioned confounding factors. Interestingly, in age and multiple covariates-adjusted analysis, we observed a strong effect on liver fibrosis of early (OR 3.038, CI 1.083-8.527, p = 0.03) only, but not of late (OR 2.291, CI 0.771-6.803, p = 0.13) menopause.

**Conclusions:** In female patients with NAFLD, menopausal status affects the risk of fibrosis severity, with a main effect of early but not of late menopause. These results, which need validation in independent cohorts, suggest that a more intensive management may be appropriate for females with NAFLD at pre- and early menopause.

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**T-20**

**PROGRESSION OF EARLY ATHEROSCLEROTIC VASCULAR DAMAGE IN PATIENTS WITH NAFLD AND IN CONTROLS OF GENERAL POPULATION DURING 10 YEARS OF FOLLOW UP**

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**Introduction:** NAFLD, the hepatic manifestation of metabolic syndrome, represent a risk factor for vascular damage. Carotid intima-media thickness (cIMT) is a known precursor to cardiovascular disease.

**Aim:** To evaluate the risk factors affecting the progression of cIMT in patients with NAFLD and in a control group from general population, the incidence of major cardiovascular events in ten years of follow up and the correlation with severity of liver damage.

**Materials and methods:** 87 patients with biopsy proven NAFLD matched 1:1 for sex and age with subjects from general population. Patients and controls were prospectively followed for a period of 10 years. In all subjects cIMT by ecocolor Doppler, ultrasonography, clinical and biochemical data were evaluated at time 0 and after 10 years follow-up. **Results:** At enrollment cIMT was significantly more elevated in NAFLD than in controls (0.87 ±0.23 vs 0.64 ±0.14, p = 0.001). Considering in the overall series (patients and controls) as increased, the value of cIMT > 0.64 mm (median of controls), at multivariate analysis the presence of steatosis, metabolic syndrome, age, BMI, ALT and glucose resulted independently associated with increased cIMT. After 10 years follow-up, 33 controls developed steatosis, while in 5 NAFLD steatosis disappeared. cIMT remained significantly more elevated in NAFLD than in controls (0.92 ±0.12 vs 0.77 ±0.15 mm, p = 0.004) but the highest average progression occurred in controls (0.07 ±0.26 and 0.13 ±0.10 mm, p = 0.04 in NAFLD and controls respectively), the progression of plaque resulted greater in NAFLD (p = 0.04).

Seven (5%) patients developed major cardiovascular events, all occurred in patients with progression of cIMT.

**Conclusion:** Our results confirm that IMT is useful in predicting future vascular events and point out the need for evaluation not only of subjects with NAFLD but also of healthy subjects for the early diagnosis of NAFLD and cardiovascular damage.

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**T-21**

**GENOTYPE 3 AND CIRCULATING SCCA-IGM ARE INDEPENDENTLY ASSOCIATED WITH HISTOLOGICAL FEATURES OF NASH IN HCV INFECTED PATIENTS**

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**Introduction:** In chronic HCV infection liver steatosis is a frequent feature, especially in genotype 3, but its clinical significance is still debated. Since SCCA-IgM immunocomplexes have been associated with more advanced liver disease and increased risk of HCC development.

**Aim:** The purpose of this study was to evaluate the occurrence of this biomarker and its possible relation with the presence of NASH at liver biopsy.

**Materials and methods:** In 91 patients with biopsy proven chronic hepatitis C serum samples were tested for SCCA-IgM by ELISA. Sera of 92 consecutive HCV negative patients with histological NAFLD were included as controls.

**Results:** SCCA-IgM was detected in 33% of HCV patients at the time of liver biopsy, but only in 4% of patients with NAFLD. In chronic hepatitis C this biomarker was found more elevated in patients with concomitant histological features of NASH and at multivariate analysis SCCA-IgM and genotype 3 were independently associated with this histologic feature. Referred to NASH, specificity and sensitivity were 97% and 44% for HCV genotype 3 vs 95% and 26% for SCCA-IgM, while PPV and PNV were 80% and 86% for the former vs 70% and 73% for the latter variable. After antiviral treatment, in HCV patients with SVR, SCCA-IgM values decreased significantly during treatment and remained persistently low after the end of therapy, while remained unchanged in the other patients.

**Conclusions:** SCCA-IgM was detectable in about one third of patients with chronic hepatitis C and its presence was significantly associated with histological NASH, independently of the infecting genotype.

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Moreover, the levels of urinary 8-iso-PGF2 were significantly higher in patients with NAFLD. The same findings were also observed after the exclusion of obese subjects, those with diabetes or with metabolic syndrome and of most of its clinical features was significantly higher in patients with NAFLD. Oxidative stress was independent from obesity, diabetes and metabolic syndrome and increased with the severity of liver steatosis evaluated by ultrasound.

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T-23

COFFEE ENHANCES THE EXPRESSION OF MITOCHONDRIAL AND ENDOPLASMIC RETICULUM CHAPERONES IN RATS WITH STEATOHEPATITIS

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Introduction and aim: Coffee consumption is inversely related with the degree of liver injury in patients with nonalcoholic fatty liver disease (NAFLD). Molecular mediators contributing to coffee beneficial effects in NAFLD remain to be elucidated.

Materials and methods: We administrated decaffeinated espresso coffee or vehicle to rats fed an high fat diet (HFD) for 12 weeks and examined the effects of coffee on liver injury by using 2D-PAGE proteomic analysis combined with mass spectrometry. Real-time PCR and western blot analysis were used to confirm gene and protein expression.

Results: Rats fed HFD + water developed panacinar steatosis, lobular inflammation and mild fibrosis, whereas rats fed HFD + coffee exhibited only mild steatosis. Coffee consumption increased liver expression of the endoplasmic reticulum chaperones Glucose-Related Protein 78 and Protein Disulfide-Isomerase A3; similarly, coffee drinking enhanced the expression of the mitochondrial chaperones Heat Stress Protein 70 and Dj-1. Furthermore, in agreement with reduced hepatic levels of 8-isoprostanes and 8-hydroxy-2′-deoxyguanosine, proteomic analysis showed that coffee consumption induces the expression of master regulators of redox status, i.e., peroxiredoxin-1, glutathione S-transferase alpha 2 and d-deoxyguanosine tautomerase.

Conclusions: In this study, we were able to identify by proteomic analysis coffee-induced ER and mitochondrial chaperones ensuring correct protein folding and degradation in the liver.

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T-24

CHANGES IN SERUM LEVELS OF ASYMMETRIC-DIMETHYLARGININE (ADMA) IN A RAT MODEL OF NON-ALCOHOLIC STEATOHEPATITIS: ROLE OF OXIDATIVE STRESS

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Background and aim: The liver plays a crucial role in the metabolism of asymmetric-dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide (NO) synthase. ADMA is metabolized...
via dimethylarginine-dimethylaminohydrolase (DDAH). This study investigated whether changes in serum levels of ADMA occur in a rat model of non-alcoholic steatohepatitis (NASH) and what is the mechanism involved.

Materials and methods: NASH was induced in Male Wistar rats by 8 weeks of feeding with an MCD diet (methionine/choline-deficient diet). Blood samples and hepatic biopsies were collected after 1, 2, 3, 4 and 8 weeks. Serum hepatic enzymes (AST, ALT and g-GT) and ADMA were evaluated. Hepatic biopsies were used for in situ NAD(P)H autofluorescence detection and for mRNA expression of DDAH and ADMA transporters (CAT-1) by RT-PCR. Tissue DDAH activity and content of lipid peroxides, glutathione and ATP were also quantified.

Results: NASH injury was confirmed by altered serum levels of hepatic enzymes. A time dependent decrease in serum ADMA levels and an increase in mRNA expression of DDAH and CAT-1 were found. The hepatic DDAH activity decreased with a concomitant increase in oxidative stress, as demonstrated by high lipid peroxide levels and low GSH content. A decrease in ATP levels and in the NAD(P)Hbound/free ratio reflecting the mitochondria alterations were detected.

Conclusions: These results indicate that the oxidative stress observed can contribute to the reduction of DDAH activity. This enzyme is a cysteine hydrolase that may be inhibited by increased reactive oxygen species associated to mitochondria dysfunction. The observed decrease in serum ADMA may be due to the increase in ADMA transporter, CAT-1. These data confirm and support the crucial role of the liver in the control of ADMA levels by taking up large amounts of ADMA from the systemic circulation. (Supported by Fondazione Cariplo, grant no. 2011-0439.)

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T-25
ENHANCER OF ZESTE HOMOLOG 2 (EZH2) EXPRESSION AND ACTIVATION IN IN VIVO AND IN VITRO NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disease in Western countries. NAFLD development/progression depends on complex interactions between genetic background, epigenetic changes and environmental factors. The trimethylation of the Lysine 27 on histone H3 (H3K27me3), by the Enhancer of Zeste Homolog 2 (EZH2) protein, is one of the most relevant epigenetic mechanisms. Although, it is well known that EZH2 activity may induce transcriptional repression of genes and microRNAs, there is no evidence of its potential expression/activity during NAFLD pathogenesis.

Aim: In the present study we investigated the EZH2 expression/activity in vivo and in vitro NAFLD and its potential correlation with liver damage.

Materials and methods: We performed biochemical and molecular analyses to evaluate EZH2 expression/activity in the liver from high-fat/high-fructose diet fed (HFa/HFr-D) rats and in free fatty acids (FFAs)-treated HepG2 cells. 3-Deazaneplanocin A (DZNep), a pharmacological inhibitor of EZH2 was used to evaluate its effects on hepatocellular damage.

Results: Our results demonstrated that EZH2 protein nuclear expression and its activity on H3K27me3 were down-regulated in livers from HFa/HFr-D fed rats and in FFAs-treated HepG2 cells. Low levels of EZH2 showed an inverse correlation with lipid accumulation and with the expression of pro-inflammatory markers, such as TNF-alpha and TGF-beta, and the expression of miR-200b and miR-155 in in vivo and in vitro NAFLD. Moreover, the use of DZNep, further increased: lipid accumulation, TNF-alpha and TGF-beta transcription, and miR-200b and miR-155 levels in FFAs-treated HepG2.

Conclusions: In conclusion we demonstrated, for the first time, that NAFLD is characterized by reduction of nuclear EZH2 expression/activity, and the treatment with DZNep may render HepG2 more susceptible to lipid accumulation and inflammation. Further investigations could be useful to clarify EZH2 role in the liver damage due to diet-induced NAFLD, as well as to develop potential targeted/preventive strategies.

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T-26
P53 STATUS AFFECTS COPPER HOMEOSTASIS IN EXPERIMENTAL MODEL OF HEPATIC STEATOSIS

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Nonalcoholic fatty liver disease (NAFLD) is a pathological condition, ranging from simple steatosis to steatohepatitis, which can progress up to hepatocarcinoma. P53 protein has been proposed as new player in NAFLD. One of its target is SCO2, a copper (Cu) chaperone essential for maintenance of Cu homeostasis, which it is implicated in the Cu secretory pathway. Cu unbalance affects lipid metabolism and seems involved in NAFLD. Through an in vitro model of steatosis we investigated the possible role of p53 status in Cu homeostasis in NAFLD.

HepG2 (wt-p53) and Huh7.5.1 (Y220C mutant p53) cells were treated for 14 and 24 h with Free Fatty Acids (FFAs), oleic and palmitic acids (2:1 ratio, final concentration 0.5 mM). Intracellular lipids and cytotoxic effects were evaluated by AdipoRed and AlamarBlue assays. Intracellular Cu content was measured by Atomic Absorption Spectrometry. mRNA and protein levels of p53, its target genes and some genes involved in copper trafficking were analysed by qRT-PCR and Immunoelectrophoresis.

In both cell lines treatment increased lipid content and was not cytotoxic. The treatment caused a different p53 response in HepG2 and Huh7.5.1. In fact, after 14 h, p53 was not affected in HepG2, whereas, in Huh 7.5.1 it was up-regulated. At 24 h, instead, we observed an up-regulation of the wt-p53 and a down-regulation of the mutated one. Phosphorylated p53 and its target p21 have the same trend of p53 in both cell lines.

In HepG2 FFAs did not affect Cu content; conversely, in Huh 7.5.1 Cu amount was reduced after 24 h. Accordingly, while in HepG2 the early activation of Cu secretory pathway is counterbalanced by p53 activation, in Huh 7.5.1, this modulation did not occur.
Our data provide new insights in p53 involvement in NAFLD pathogenesis, highlighting its role in the modulation of copper homeostasis.

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T-27

INHIBITION OF INDOLEAMINE 2,3-DIOXYGENASE AMELIORATES INFLAMMATION AND FIBROSIS IN EXPERIMENTAL STEATOHEPATITIS

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Background/aims: The pathogenic mechanisms underlying development of nonalcoholic steatohepatitis are still elusive. Indoleamine 2,3-dioxygenase (IDO), an intracellular enzyme that mediates the catabolism of L-tryptophan to L-kynurenine, plays an important role in hepatic immune regulation, mediating inflammation or tolerance depending on the injury and the tissue. In the present study, we examined the effect of IDO inhibition on steatohepatitis induced by a methionine and choline deficient (MCD) diet in mice.

Materials/methods: Balb/C mice fed a MCD diet for 8 weeks, were treated with the specific IDO inhibitor, 1-methyl-d-triptophan (1MT, 5 mg/ml) or its vehicle in drinking water. Plasma kynurenine levels were evaluated by isocratic reverse phase chromatography. Histology was analyzed by H&E or Sirius red staining, followed by a semiquantitative score. Intrahepatic gene expression was assayed by quantitative real time PCR.

Results: MCD diet increased IDO activity, as indicated by plasma levels of kynurenine. 1MT administration caused a significant reduction of IDO activity in the liver, and markedly reduced ALT levels in MCD-fed mice. In addition, histologic parameters of inflammation were significantly ameliorated. This effect was associated with reduced intrahepatic levels of proinflammatory factors, including CD11b, CCL2, TNF, and IL-1 beta. Fibrosis induced by the MCD diet was decreased by 1MT co-administration, together with reduced intrahepatic gene expression of TGF-beta and alpha-SMA.

Conclusions: IDO inhibition by 1MT exerts a protective action in MCD-induced hepatic inflammation and fibrosis.

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T-28

INCIDENCE AND RISK FACTORS FOR EXTRA-HEPATIC MALIGNANCIES IN PRIMARY BILIARY CIRRHOSIS: A COMPARATIVE STUDY FROM TWO EUROPEAN REFERRAL CENTERS

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Introduction: There are limited information and divergent results on the prevalence/incidence, survival, and risk factors for developing EM in PBC.

Aim: To analyze the incidence/prevalence, risk factors and survival for EM in PBC patients from two European centers.

Methods: The study was carried out in two series of PBC patients from two European centers (361 of Padova, Italy and 397 of Barcelona, Spain) followed-up for a mean period of 7.7 ± 7 years and 12.2 ± 7 years respectively. The incidence of EM was compared to the estimated incidence data from IARC (International Agency for Research on Cancer). Demographic features and factors associated with tumor development (gender, age, alcohol consumption, smoking habit, familial predisposition) were recorded. Survival analysis was compared with the expected survival predicted by the Mayo model.

Results: 72 patients (35 from Padova and 37 from Barcelona) developed EM. The prevalence of cancer was similar in Padova (9.7%) and Barcelona (9.4%). The incidence of EM was also similar (855.01 vs 652.86 per 100,000 patient-year respectively, \( p \) = n.s. [95% CI −0.002 to 0.006]). The overall incidence of EM in the study population was similar to the expected incidence in the same geographical area (observed/expected ratio = 1.18 [95% CI 1.0–1.2]). Older age was the only factor associated with the development of EM. When analyzing the two series separately, familial predisposition was associated with higher likelihood of EM in Padova. Survival was similar in those with either or without EM (29.2 and 33.4 years respectively, \( p \) = n.s.). The actual survival was similar to that predicted by the Mayo model.

Conclusions: The prevalence/incidence of EM is similar in Italy and Spain and is not different from the general population. Older age is the only risk factor associated with EM in PBC. The occurrence of cancer during the follow-up does not influence the natural history of liver disease.

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T-29

HUMAN BILIARY TREE STEM/PROGENITOR CELLS (HBTS Cs) FROM PERIBILIARY GLANDS (PBGS) OF ADULT LIVER DISPLAY IMMUNOMODULATORY PROPERTIES THROUGH FAS/FAS LIGAND INDUCED T-CELL LYMPHOCYTE APOPTOSIS

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Introduction: hBTSCs have the potential for regenerative medicine in liver and pancreas diseases. T-cell control was recently demonstrated for mesenchymal stem cells.

Aim: The aims of this study were to evaluate Fas-L expression within the stem cell niches of adult biliary tree (PBGs), and to study the interaction between hBTSCs and human lymphocytes.

Materials and methods: HLA antigens, Fas and Fas-L expression were evaluated by immunofluorescence and Western blotting (WB) in cells of human biliary tree in comparison with fibroblast cells, dental pulp stem cells and bone marrow mesenchymal stem cell. The influence of hBTSCs on lymphocytes’ activation and apoptosis were assessed by co-culturing experiments.

Results: Adult hBTSCs expressed both class I and class II HLA antigens, whereas fetal hBTSCs only class I HLA antigens. 10 to 30% of the hBTSCs in PBGs were positive for Fas-L. Fas-L+ cells were mostly located at the bottom of PBGs and co-expressed EpCAM (Epithelial-Cell-Adhesion-Molecule) and proliferation marker (PCNA: Proliferating-Cell-Nuclear-Antigen). Mature cells at the bile duct surface epithelium (mature cholangiocytes) were almost all negative for Fas-L. In culture experiments, confocal microscopy demonstrated that Fas-L expression was restricted to FcAM+ cell (a marker associated with endodermal stem cells) hBTSCs. WB confirmed that hBTSCs constitutively expressed high level of Fas-L which increased after co-culture with T-cells. Fluorescence-activated cell sorting (FACS) analysis revealed that activated CD4+ and CD8+ T-cells co-cultured with hBTSCs underwent to a massive induction of apoptosis. Fas receptor appeared over-expressed in T-cells co-cultured with hBTSCs respect to resting T-cells.

Conclusions: Our data demonstrated that hBTSCs can induce "premature" apoptosis in T-cells trough the activation of Fas/Fas-L pathway.

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T-30

SEX HORMONES DIFFERENTLY REGULATE HEPATIC HEPcidin EXPRESSION AND SYSTEMic IRON HOMEostasis IN VIVO

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Introduction: Iron loading has been associated with the progression of several liver diseases. Moreover, a number of studies indicate that female and male exhibit differences in the progression of chronic liver diseases, including hereditary hemochromatosis. Better understanding of the mechanisms associated with gender-related differences in iron homeostasis may help to develop new therapeutic strategies in hepatic diseases. Heparicin, a small hormone synthesized mainly by the liver, is the central regulator of iron homeostasis. The BMP(6)-SMAD signaling cascade is the main pathway involved in hepcidin expression regulation. In humans and animal models, gender influences hepcidin and body iron levels. So far data on the role of sex hormones in the control of hepcidin transcription and iron homeostasis have been controversial.

Aim: To study the effects of androgens and estrogens on the regulation of hepcidin expression and systemic iron status in vivo.

Materials and methods: Male and female mice underwent orchiectomy or ovariectomy. Furthermore, male and female mice were parenterally treated with androgens or estrogens.

Results: Orchietomized mice showed higher hepcidin transcription, a significant activation of the BMP6-SMAD pathway, and lower liver iron content in comparison to sham-operated mice. This was not the case for ovariectomized mice. Yet, androgens administration led to a 50% decrease of hepcidin mRNA and significant spleen iron depletion. In contrast, estrogens administration, while decreasing hepcidin transcription, was associated with spleen iron retention.

Conclusions: Our data indicate that both male and female sex hormones control hepatic hepcidin expression; estrogens seem to act on systemic iron status not only via direct inhibition of hepcidin transcription but also through a non-hepcidin driven effect involving tissue iron re-distribution.

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T-31

SIRT1 ACTIVITY IN HEPATIC STELLATE CELLS DURING LIVER INJURY IS A TARGET FOR THE MODULATION OF THE FIBROGENIC PROCESS

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Introduction: Hepatic stellate cells (HSC) represent 15% of the total resident cells in normal liver. Following liver injury, HSC transform into proliferating profibrogenic myofibroblasts. The activation of SIRT1, a NAD-dependent histone deacetylase, is correlated
with prosurviving phenotype in many cell lines, and it is normally expressed in liver.

**Aim:** We investigated the role of SIRT1 activation in HSC during liver injury as a possible therapeutic target for liver fibrosis.

**Materials and methods:** We evaluated the expression of SIRT1 in human and mouse HSC and its role in response to stress conditions. We also tested the in vivo effects of SIRT1 modulation in different mouse models of liver injury (carbon tetrachloride exposition, bile duct ligation, high fat diet).

**Results:** We found that SIRT1 is constitutively expressed in human and mouse HSCs; its activation reduces proapoptotic effects of hydrogen peroxide in HSC. Treating the cells with the SIRT1 inhibitor EX-527 resulted in increased apoptosis and reduced activation during oxidative stress related damage. In the in vivo models EX-527 treated animals showed decreased level of HSC activation as assessed by qPCR and reduced collagen deposition.

**Conclusions:** SIRT1 inhibition in HSC increases stress-related apoptosis and decreases cell activation in vitro and decreases the number of activated and proliferating HSC in the animal model of liver injury. The inhibition of SIRT1 could therefore prevent the fibrogenic alteration of the parenchyma during liver injury.

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T-32

BLEEDING-UNRELATED MORTALITY IS NOT INCREASED IN PATIENTS WITH CIRRHOSIS AND ASCITES ON TREATMENT WITH β-BLOCKERS: A META-ANALYSIS

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**Introduction:** Non-selective β-blockers have been hypothesized to have harmful role on survival in patients with cirrhosis and ascites.

**Aim:** To evaluate bleeding-unrelated mortality in patients with cirrhosis on treatment with β-blockers for primary and secondary prophylaxis of gastric/variceal bleeding.

**Materials and methods:** A systematic review of the literature was performed by analyzing randomized controlled trials (RCT) from 1990 to 2012 on primary and secondary prophylaxis with β-blockers for gastric or variceal bleeding in cirrhotics. For each study we evaluated mean drug dose, prevalence of ascites, overall mortality and causes of death; drop outs were excluded from analysis. Secondary prophylaxis studies were divided in two subgroups using β-blockers mean dosage 120 mg/day as cut-off. Thus we performed a meta-analysis using bleeding-unrelated mortality as endpoint in all the studies, using the random effect model.

**Results:** Twenty-three on primary and 28 RCT on secondary prophylaxis were included. A total of 4481 patients were included in meta-analysis, 1784 had ascites. In primary prophylaxis 215/955 patients died for bleeding-unrelated causes, in a proportion not different between those who were or were not on treatment with β-blockers (OR 0.91, 95% CI 0.73–1.15). In secondary prophylaxis RCT bleeding-unrelated deaths were not higher in patients on treatment with β-blockers (189/1143 vs 225/1208; OR 0.90, 95% CI 0.67–1.23), even if there was significant heterogeneity amongst studies. These data were confirmed when subgroup analysis was performed in those RCT using dose of β-blockers lower or higher than 120 mg/day (134/732 vs 158/764, OR 0.87, 95% CI 0.59–1.28, 2 42% and 48/374 vs 57/309, OR 1.01, 95% CI 0.55–1.84, 2 34% respectively).

**Conclusions:** In RCT there was no correlation between the use of β-blockers and increased prevalence of bleeding-unrelated mortality. Therefore, the absence of a harmful role in patients with cirrhosis and ascites can be hypothesized.

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T-33

RESISTANCE TO THROMBOMODULIN IS ASSOCIATED WITH DE NOVO PORTAL VEIN THROMBOSIS AND COULD BE A MODIFIABLE FACTOR TO IMPROVE PROGNOSIS IN CIRRHOSIS

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**Introduction:** Portal vein thrombosis (PVT) is a frequent event in liver cirrhosis. Plasma from patients with cirrhosis shows an intrinsic resistance to thrombomodulin (TM) activity as demonstrated by in vitro tests for Endogenous Thrombin Potential with/without TM (ETP-ratio).

**Aim:** Exploring retrospectively the potential impact of TM-resistance on the rate of de novo PVT and the natural history of cirrhosis.

**Materials and Methods/Results:** Sixty-five patients with cirrhosis tested for ETP with/without TM. Clinical, endoscopic variables, presence/absence of PVT by Doppler-US and/or TC examination were collected at basal evaluation and up to 4 years. The incidence of de novo PVT was the primary clinical end-point. We also considered transplantation free-survival. ETP-ratio upper than the 95 percentile of 173 healthy-controls defined TM-resistance. ETP-ratio was not different by comparing patients with TM-resistance on the rate of de novo PVT and the natural history of cirrhosis. Among no-PVT patients, 11 developed de novo PVT in the follow-up. The incidence of PVT was higher in those patients with TM-resistance (n = 36) also after adjustment for Child-score (HR: 7.68; 90% CI: 1.32–44.54, p = 0.017). Seventeen patients experienced at least one PHT-related complication and 23 patients died or were transplanted. The mean survival time-free of transplantation was 2.7 vs 3.6 years by comparing, respectively, patients with vs without TM-resistance (p = 0.005, log-rank test). However, only Child-score independently predicted transplantation free-survival.

**Conclusions:** The occurrence of PVT should be explained, in part, by the pro-coagulant imbalance described in patients with advanced liver disease. TM-resistance could be a potential modifiable factor to improve survival in patients with cirrhosis.

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T-34

CHRONIC TREATMENT WITH BETABLOCKERS
FOR THE MANAGEMENT OF PORTAL HYPERTENSION: A MULTI-CENTER CROSS-SECTIONAL STUDY IN ELDERLY PATIENTS WITH CIRRHOSIS

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Introduction: Co-morbidity is frequent in elderly patients and may affect therapy because of drug interactions and/or different indications/contraindications. In cirrhosis the target of heart rate below 60-bpm under non-selective beta-blockers (NSBBs) protects by bleeding due to portal-hypertension.

Aim: Prospectively assessing the safety and the applicability of beta-blockers (BBs) for portal hypertension in >65 years old in-patients with cirrhosis.

Materials and methods/Results: One-hundred-eighty-four cirrhotic patients admitted in 38 Italian internal medicine wards during 4 weeks of 2008 were included in the REPOSI study. Pharmacological history, length of hospital stay (LOS), and occurrence of clinical events during hospitalization were recorded prospectively. Uni- and multivariable analysis tested the associations of BBs taken for portal hypertension with the clinical outcome. Sixty-four patients (35%) were receiving BBs at admission (50% NSBBs). The main indication was portal hypertension in 41% of patients, however in only 5 heart rate was <60 bpm. LOS was 8.6 ± 6.3 days for patients not-receiving BBs, 10.4 ± 6.3 for patients receiving BBs for portal hypertension, and 13.8 ± 12.2 for patients receiving BBs for cardiovascular disease (p = 0.016). Twenty-five patients had kidney failure (KF) associated with BBs (p < 0.01). Adjusting for factors independently associated to KF, this association was no more significant, whereas older age and PPI use were independently associated with KF.

Conclusions: Up to 50% of elderly in-patients with diagnosis of cirrhosis receive BBs as a prophylaxis of variceal bleeding, but the target of low heart rate is rarely achieved. In such patients BBs are associated with longer LOS and KF but no potential cause–effect relationship was found in our series. Further studies are warranted to identify a correct approach to the use of BBs in elderly patients with cirrhosis.

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T-35

ISCHEMIA MODIFIED ALBUMIN (IMA) FOR THE DIAGNOSIS OF BACTERIAL INFECTION IN HOSPITALIZED PATIENTS WITH CIRRHOSIS

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Background/Aims: Human serum albumin (HSA) carries many non-oncotic functions, such as free radical scavenging and detoxification. The N-terminal site binds transition metal ions, thus acting against oxidative stress. Clinical conditions characterized by a pro-inflammatory state, including acute-on-chronic liver failure, have been found to impair this specific activity, which can be easily assessed by measuring the circulating level of ischemia-modified albumin (IMA). Thus, this study aimed to assess whether IMA can be considered a marker of bacterial infections in patients with cirrhosis.

Methods: 133 cirrhotic patients (Child-Pugh A/B/C: 27/77/29) hospitalized for acute complications of the disease and 50 age- and sex-matched healthy controls were enrolled. Clinical and biochemical data were assessed at admission. The serum IMA level was measured by using the ACB test. Bacterial infection was diagnosed in 41 patients according to microbiological and clinical data.

Results: IMA concentration was significantly higher in cirrhotic patients than in controls (0.51 ± 0.12 vs 0.39 ± 0.12, p < 0.001), but no correlations were found with MELD and Child-Pugh scores. In contrast to all other complications, IMA was greater in infected than non-infected patients (0.58 ± 0.12 vs 0.48 ± 0.10, p < 0.001). ROC curve analysis showed that IMA had the same discriminatory performance for bacterial infection as C-reactive protein (CRP). At the logistic regression, the parameters independently associated with bacterial infection were IMA, CRP, serum sodium and INR. Using these parameters, we developed a novel score (9.852 × IMA + 0.181 × CRP + 1.426 × INR − 0.143 × Na) showing a greater diagnostic performance (AUROC = 0.83) than either IMA or CRP alone.

Conclusions: Elevation of circulating IMA is associated with bacterial infection in cirrhosis. IMA showed a diagnostic accuracy for bacterial infection similar to that of CRP, while a score including IMA, CRP, serum sodium and INR enhanced the diagnostic performance of either IMA and CRP alone. These data prompt further investigation to assess the feasibility of IMA as a novel test to diagnose bacterial infection in the clinical ground.

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ENDOCANNABINOID-RELATED MOLECULE OLEOYL-ETHANOLAMINE (OEA) CORRELATES WITH CLINICAL COMPLICATIONS AND 1-YEAR SURVIVAL

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Background/Aims: Anandamide (AEA), the most studied endocannabinoid (EC), has been implicated in the pathogenesis of chronic liver disease. More recently, two EC-related molecules, namely oleoyl-ethanolamine (OEA) and palmitoyl-ethanolamine (PEA), have been reported to be elevated in patients with cirrhosis. Thus, we aimed to assess in a large cohort of cirrhotic patients whether circulating PEA and OEA are associated to specific clinical features of the disease.

Materials and methods: 156 cirrhotic patients (mean age 60.3 ± 11.5; 66% male) hospitalized for an acute complications of the disease and 108 healthy subjects (mean age 59.6 ± 11.2 years; 65% male) were enrolled. Clinical and biochemical parameters were recorded and patients were followed up until 1-year. Plasma levels of AEA, PEA and OEA were measured through LC/MS/MS.

Results: Circulating AEA, OEA, and PEA were all significantly increased in cirrhotic patients as compared to healthy subjects. As female present higher circulating EC than male in both healthy and increased in cirrhotic patients as compared to healthy subjects. As

Conclusions: Circulating levels of AEA, OEA and PEA are increased in patients with cirrhosis. However, only the OEA level is associated with specific clinical complications of cirrhosis and performs as an independent predictor of survival. As OEA has been found to induce vasorelaxation in the mesenteric artery of healthy rats by activating the vanilloid receptor thus also potentiating the AEA activity, its role in the pathophysiology of the hemodynamic complications developing in advanced cirrhosis deserves further investigation.

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ADHERENCE TO A MODERATE SODIUM RESTRICTION DIET IN OUTPATIENTS WITH CIRRHOSIS AND ASCITES: A REAL LIFE CROSS-SECTIONAL STUDY

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Background: A moderate sodium restriction diet should be indicated in patients with cirrhosis and ascites. Nevertheless, there is a lack of specific investigation on its correct application.

Aims: To evaluate the adherence of patients with cirrhosis and ascites to a moderately low-salt diet and the impact on total calories intake and serum sodium concentration.

Methods: 120 outpatients with cirrhosis and ascites were interviewed with a pre-established questionnaire. A quantitative assessment of nutrient and salt intake was performed.

Result: A moderately low-salt diet was followed by 37 patients (group A). Of the 83 patients that didn’t follow the diet (group B), 54 thought that they were following it. The mean daily sodium was 79.5 ± 5.5 mmol/day (group A) and 205.9 ± 14.1 mmol/day (group B), p < 0.0001. The adherence to diet was related to the severity of cirrhosis, and was higher among candidates to liver transplantation and in patients followed through the Care Management Program. Patients of Group A had reduced the mean daily calorie intake by 20% compared with Group B patients (p < 0.0005), while there was no difference on the occurrence of hyponatremia.

Conclusions: The study shows a poor adherence of patients with cirrhosis and ascites to a moderate dietary sodium restriction. Adherence to a diet seems to increase with the worsening of liver disease, probably due to the reduction of alternative therapeutic options. In addition, a deficiency in the educational process can lead the patient to follow a sodium-reduced diet by means of dangerous tools such as reducing the overall daily food intake.

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SVR IS ASSOCIATED WITH NO RISK REDUCTION OF HCC DEVELOPMENT IN PATIENTS WITH HCV-RELATED CIRRHOSIS. A PROSPECTIVE, UP-TO 23 YEARS, COHORT FOLLOW-UP STUDY

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Introduction and aims: Retrospective studies showed that the achievement of sustained virological response (SVR), in the
short-term, reduces/delays but not abolishes the risk of hepatocellular carcinoma (HCC) in HCV-related compensated cirrhosis. Whether this is true, in the long-term, is still unknown.

**Methods:** All consecutive HCV-related cirrhotic patients enrolled between 1989 and 1992 who received IFN-based therapy at three referral centers in Milan area were included. Clinical and ultrasound surveillance was carried-out at six-months intervals. HCC diagnosis was determined, over the years, according to updated guidelines. Rates of HCC in non-SVR and in SVR patients were calculated dividing the number of HCC by the number of patient-years at risk collected in the two groups. Curves of the cumulative incidence of HCC were drawn considering competing causes of liver-related decompensation and death. Univariate and multivariate Cox regression analysis, adjusted for age, sex, alpha feto-protein, albumin, HCV genotype were carried to assess the effect of SVR on HCC risk.

**Results:** Among 194 patients (106 males, mean age: 55 years), 38 achieved SVR: 12/132 (9%) infected by HCV genotype 1 and 19/52 (37%) by HCV genotype 2. After a median follow-up of 14.5 years (range 0.1–23.4 years), 64 patients developed HCC. The rate of HCC was higher in non-SVR compared to SVR: 56/156, 2.7/100-year (2.1–3.5) and 8/38, 1.4/100-year (0.7–2.7), respectively (p = 0.02, HR (95% CI) = 0.42 (0.20–0.89)). However, no difference was found between the two groups at multivariate analysis (HR (95% CI) = 0.56 (0.24–1.31)).

**Conclusions:** In HCV-infected patients, SVR was not associated with risk reduction of HCC development if cirrhosis has already occurred. Therefore, effective antiviral treatment should be recommended at early disease stage.

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**T-39**

**DOES THE GUT MICROBIOTA MODULATE THE INFLAMMATORY STATE IN CIRRHOTIC HOST?**

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**Introduction:** In cirrhotic patients an alteration in gut microbiota has been characterized by an overgrowth of Enterobacteriaceae (i.e. *E. coli*) and a decrease in autochthonous familiae such as the Clostridiaeae (i.e. *Faecalibacterium prausnitzii*). This dysbiosis has been recently associated with systemic complications, such as hepatic encephalopathy.

**Aim:** We aimed to correlate the microbiome features with cirrhosis and the pro-inflammatory condition in these patients.

**Materials and methods:** Temporal temperature gradient gel electrophoresis (TTGE) was performed on stool specimens of cirrhotics and age-matched controls. The *F. prausnitzii/E. coli* ratio, assessed by quantitative PCR, was used to define gut dysbiosis. TTGE patterns were compared by means of *R*², while qPCR results were compared through Mann–Whitney *U* test. The importance of variables in defining SIRS status was computed with a custom R module, and tested with Pearson's correlation coefficient. The diagnosis of SIRS was made according to guidelines.

**Results:** Twenty-seven cirrhotics (17 Child B/C) and nine controls were included. Cirrhotic faeces, compared to controls, showed different TTGE profiles and higher levels of *E. coli* (*P* < 0.05), while controls had higher levels of *F. prausnitzii* (Fig. 1). Within the cirrhotic group, *E. coli* levels showed a positive correlation with C-reactive protein (*r² = 0.54; *P* < 0.05), erythrocyte sedimentation rate (*r² = 0.48; *P* < 0.05) and cardiac frequency (*r² = 0.58; *P* < 0.05). Ten cirrhotics were diagnosed to have a SIRS. Logistic regression showed a trend to significance between SIRS and dysbiosis (CI: 0.8–1.7; *P* = 0.06).

**Conclusion:** Cirrhosis is associated with significant alterations in stool microbiome. Specific bacterial imbalance, expressed by the *F. prausnitzii/E. coli* ratio, is associated with signs of the pro-inflammatory condition present in cirrhotic patients.

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**T-40**

**PATHOGENESIS OF SOLUTE-FREE WATER RETENTION IN EXPERIMENTAL ASCITIC CIRRHOSIS: IS VASOPRESSIN (ADH) THE ONLY AGENT TO BLAME?**

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**Background:** Catecholamines and angiotensin II overproduction reduces fluid delivery to the Henle’s loop and renal excretion of solute-free water. In ascitic cirrhosis, hypersecretion of vasopressin (ADH) is thought to rule tubular free-water reabsorption (TFWR), but ADH V2-receptor antagonists are not beneficial in the long-term treatment of hyponatremic ascites.

**Aim:** We explore the hypothesis that excess TFWR in ascitic cirrhosis could depend on proximal tubular fluid retention rather than on ADH hypersecretion.

**Methods:** 60 ascitic cirrhotic rats were carefully assessed: rats receiving 13-week CCl4 administration only (group G1), cirrhotic rats receiving daily diuretics (0.5 mg/kg furosemide + 2 mg/kg K+-canrenoate during the 11th–13th weeks of CCl4) (G2), cirrhotic rats treated with diuretics + SSP-004240F1, receptor agonist and sympatholytic agent, 2 (G3), 7 (G4), or 10 mg/kg (G5); ascitic rats treated with diuretics + guanfacine 2 mg/kg (in G3) reduced TFWR from 32 ± 8 to 12 ± 2 L/min, respectively (*P* < 0.05). In the population of 60 rats, TFWR did not correlate with plasma ADH levels (*r* < 0.05), but showed correlations with plasma levels of *E. coli* (*P* < 0.03) and cardiac frequency (*r* = 0.87; *P* < 0.03).

**Results:** Diuretics + V2 antagonists (in G6) and diuretics + guanfacine 2 mg/kg (in G3) reduced TFWR from 32 ± 11 (in G1) to 21 ± 9 and 20 ± 8 L/min, respectively (*P* < 0.03). Compared to G2, the addition of guanfacine (2 mg/kg) (in G3) to diuretics reduced serum norepinephrine from 423 ± 122 to 211 ± 111 ng/L (*P* < 0.01), plasma renin activity from 25 ± 12 to 9 ± 7 ng/mL/h (*P* < 0.03), and TFWR from 45 ± 18 to 20 ± 8 L/min (*P* < 0.01). In the population of 60 rats, TFWR did not correlate with ADH levels (*r* = 0.12; *P*: n.s.), but showed correlations with plasma...
Conclusions: Furosemide exacerbates both secondary aldosteroneism and TFWR. Increased distal delivery of fluid, achieved through a reduction in adrenergic function and renin levels, is as effective as ADH V2-receptor blockade to blunt excess TFWR in ascitic cirrhosis.

Introduction: Metabolic syndrome (MS) has become one of the major causes of liver disease in developed countries. Still, data are scarce on the results of surgical treatment of hepatocellular carcinoma (HCC) occurring as a complication of MS.

Aim: To evaluate the epidemiological trend in a tertiary surgical referral Center and the outcomes of liver transplantation (LT) and liver resection (LR) in patients affected by MS as unique oncological risk factor.

Materials/Methods: In our Center, from January 1997 to April 2013, 363 and 685 patients with HCC underwent LR and LT, respectively. Among them, 45 patients had MS as only risk factor for developing HCC (MS-HCC): 28 were treated with LR and 17 with LT. Data were collected retrospectively.

Results: There was an increasing prevalence of MS-HCC: from 0% in the first two-year interval (1997–1998) up to 7% in the most recent years (2011–2012), with an expectation of nearly 10% for year 2013. Transplanted patients showed higher probability of developing postoperative complications (88% vs 50%), with major complications (Dindo-Clavien grade 3 or more) occurring in 35.3% in the LT group and in 10.7% in the LR group. While all 17 LT patients had a cirrhotic liver, only 3 LR patients were cirrhotic; in particular, 68% (19/28) LR patients presented only minor fibrosis or even no structural change. The 5-year patient survival was similar in the two groups (83.7% for LT and 80.1% for LR). Recurrences occurred only in the LR group, with a 5-year recurrence-free survival of 60% and a median survival time of 6.5 years.

Conclusions: MS-HCC is increasing in surgical referrals, and it can occur also in livers with no or slight alterations. Care must be concentrated in the postoperative phase, as comorbidities fostered by MS may favour major complications. Despite this, surgical treatments offer satisfying results.

Table 1 shows the key stratifications of PSS. Criteria for second line protocol were met in 102 patients (39%): in these, PSS was 7.1 (5.4–8.9) vs 3.1 (2.3–3.9) months in those who did not met inclusion criteria.

<table>
<thead>
<tr>
<th>Patients (N)</th>
<th>Median survival, months (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (260)</td>
<td>4.1 (3.3–4.9)</td>
</tr>
<tr>
<td>BCLC</td>
<td></td>
</tr>
<tr>
<td>B (21)</td>
<td>9.3 (4.0–14.6)</td>
</tr>
<tr>
<td>C (193)</td>
<td>4.7 (3.7–5.6)</td>
</tr>
<tr>
<td>D (46)</td>
<td>1.8 (1.4–2.2)</td>
</tr>
<tr>
<td>Child–Pugh</td>
<td></td>
</tr>
<tr>
<td>A (135)</td>
<td>6.4 (4.7–8.1)</td>
</tr>
<tr>
<td>B (110)</td>
<td>3.6 (2.6–4.6)</td>
</tr>
<tr>
<td>C (15)</td>
<td>1.6 (1.2–1.9)</td>
</tr>
<tr>
<td>Second-line trials</td>
<td></td>
</tr>
<tr>
<td>“Criteria unmet” (158)</td>
<td>3.1 (2.3–3.9)</td>
</tr>
<tr>
<td>“Criteria met” (102)</td>
<td>7.1 (5.4–8.7)</td>
</tr>
<tr>
<td>Reasons for sorafenib discontinuation</td>
<td></td>
</tr>
<tr>
<td>Tumor progression (133)</td>
<td>4.1 (3.0–5.2)</td>
</tr>
<tr>
<td>Adverse events (87)</td>
<td>7.1 (5.3–8.9)</td>
</tr>
<tr>
<td>Liver function deterioration (40)</td>
<td>1.8 (1.5–2.1)</td>
</tr>
</tbody>
</table>
**Conclusion:** The survival of patients who discontinued sorafenib is influenced by both tumor progression pattern and liver impairment, key factors in prognostic prediction and design of second-line trials.

http://dx.doi.org/10.1016/j.jdd.2014.01.082

T-43

THE RED CELL DISTRIBUTION WIDTH (RDW) IS A NOVEL POWERFUL, INEXPENSIVE PREDICTOR OF SURVIVAL FOR PATIENTS WHO PRESENT WITH HEPATOCELLULAR CARCINOMA

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**Background:** It has been shown recently that the red cell distribution width (RDW; SD of red cell volume/mean cell volume × 100) is a strong prognostic marker of survival among patients with neoplastic and non-neoplastic diseases, possibly because it may reflect inflammation. The aim of this study was to identify whether RDW is a prognostic index for hepatocellular carcinoma (HCC).

**Methods:** 205 consecutive Caucasian patients with HCC (153 males; 198 with cirrhosis; 95 with hepatitis C and 19 with hepatitis B) diagnosed at an Academic Center in Northern Italy from November 2003 to September 2013 were studied. The status of all patients was known and documented up to (and including) the censor date of 25 October 2013. RDW at diagnosis was analyzed in relationship to mortality by any cause.

**Results:** Median survival was 782 days (95% CI: 594–1049). Median RDW was 14.5% (95% CI: 14.3–14.8). 131/205 patients (64%) had a RDW ≤15% (median, 13.9, 95% CI 13.7–14.3; group A), and 74/205 (36%) a RDW >15% (median 16.2, 95% CI 15.9–16.6; group B). In group A, median survival was 1154 days (95% CI: 809–1368) vs. 298 days (95% CI: 233–651) in group B, hazard ratio 2.45 (95% CI: 1.64–3.79), p < 0.0001. On a Cox proportional hazard model including age (>70 or >70 years), sex, BCLC stage (A–D), tumor size (<2, 2–5 or >5 cm), and AFP (<200 or >200 ng/ml), a RDW >15% was a strong independent predictor of survival (hazard ratio 2.49, 95% CI: 1.64–3.80).

**Conclusion:** RDW is a novel powerful, inexpensive biomarker to predict survival of HCC patients at presentation.

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T-44

HEMOSTATIC STATUS AND PORTAL VEIN THROMBOSIS (PVT) IN CIRRHOTIC PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC)

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**Background and aim:** Studies exploring the hypercoagulable state induced by HCC and its correlation with the risk of PVT are lacking. The aim of the present study was to evaluate the thrombophilic role of HCC as risk factor for PVT development.

**Methods:** Cirrhotic patients with and without HCC were prospectively enrolled. Age- and sex-matched healthy individuals constituted the control group for thromboelastometry (ROTEM). All patients underwent: ROTEM, platelet count, determination of prothrombin time and of levels of pro and anticoagulation factors. During follow-up, PVT onset was recorded.

**Results:** 76 cirrhotics, 41 with HCC, and 48 healthy controls were included. Volume of active HCC was >5 cm³ in 18 patients. Levels of pro and anticoagulation factors were similar between patients with and without HCC, but fibrinogen was increased in HCC patients with active volume >5 cm³ HCC compared to those with ≤5 cm³ HCC (348.72 ± 124.06 mg/dL vs. 237.64 ± 99.18 mg/dL) and to cirrhotics without HCC (260.57 ± 126.07 mg/dL) (p = 0.006). Platelet count was significantly increased in HCC compared to non–HCC, and this was especially true in Child Class A patients. Patients with HCC showed significantly lower clotting time and maximum clot formation at ROTEM compared to controls. The hypercoagulable state was present even when HCC patients were compared to cirrhotics without HCC, especially in Child A patients, with statistically significant differences in MCF EXTEM/NATEM. One year- incidence of PVT was 19.5% (8/41) and 5.7% (2/35) in HCC and non-HCC patients, respectively (p = 0.04). In the HCC group, 4/8 portal vein thromboses occurred in patients in Child A group. Fibrinogen test of ROTEM, MCF and AUC were statistically greater in HCC patients who later developed PVT.

**Conclusions:** Cirrhotics with HCC demonstrate a prothrombotic hemostatic balance resulting in an increased risk of PVT. This prothrombotic state seems to be detectable by ROTEM and thus possibly suggest those who could benefit from thromboprophylaxis.

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CONCOMITANT VERSUS SEQUENTIAL TREATMENT WITH TACE AND SORAFENIB IN HCC PATIENTS

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Background: A therapeutic strategy based on the concomitant – rather than sequential – administration of transarterial chemoembolization (TACE) and sorafenib for the treatment of patients with hepatocellular carcinoma (HCC) has gained particular interest in recent years. In this field-practice study, we compare the clinical outcomes associated with the concomitant and the sequential strategy, in HCC patients included in the Nation-wide Italian database ITA.LI.CA.

Patients and methods: The ITA.LI.CA. database contains data of 5136 HCC patients treated at 18 Italian Centers. All patients treated with TACE and sorafenib, either sequentially or in combination, were included in this analysis. The following endpoints were considered: overall survival (OS), time to progression (TTP) and disease control rate (DCR). Data reported in patients who received concomitant treatment were compared with those observed in patients on sequential treatment.

Results: In total, 128 patients were observed (98 males; age 44.7 years; 107 in BCLC-B stage). Of these, 17 had received concomitant treatment with TACE and sorafenib, whereas the sequential strategy was applied to 111 subjects.

Median OS was significantly longer in patients on concomitant treatment than in those who received the sequential strategy (25 months vs 12 months; p < 0.01). A similar finding was observed for TTP (6 months vs 4 months; p < 0.05) and for DCR (30% vs 25%; p < 0.05). One patient treated with the concomitant strategy and two patients on the sequential therapy experienced a complete response. No relevant safety warnings were reported.

Conclusion: Although the different sample size of the two groups hampers the analysis, the results of this large, field-practice database study lend some support to the effectiveness and safety of sorafenib in combination with TACE rather than after the failure of this loco-regional therapy.

http://dx.doi.org/10.1016/j.dld.2014.01.085

CHOLANGIOCARCINOMA (CCA) EXPRESS CHEMOKINE RECEPTOR CXCR7: IMPORTANT ROLE OF CXCR7 IN MEDIATING CXCL12 INDUCED CCA CELLS CHEMOTAXIS AND SURVIVAL

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Background/Aims: Migration, invasion and metastasis of CCA cells are dependent on signals generated by stromal cells, including myofibroblastic hepatic stellate cells (HSC). We recently showed that HSC produce CXCL12, a chemokine which binds two different receptors, CXCR4 and CXCR7. CXCR7 has been shown to act either as a scavenger of CXCL12, or to start intracellular signaling alone or in cooperation with CXCR4. The purpose of the present study was to investigate the expression and function of CXCR7 in CCA cells.

Methods: CXCL12 expression and signaling pathways were investigated by Western blotting, siRNA against Knock-down of CXCR7 and beta arrestin-2 was performed by siRNA. Migration of CCA cells was assayed using modified Boyden chambers, and cell survival was evaluated by MTT. Immuno-histochemistry was conducted to evaluate the expression of CXCR7 in human specimens of CCA.

Results: CXCR7 was expressed by CCA cells in human tissues and by CCA cell lines in vitro. CCX733, a specific antagonist of CXCR7, inhibited CXCL12-induced HuCCT-1 migration and survival, without affecting CXCL12-induced phosphorylation of ERK1/2 and AKT. Similarly, in CXCR7-depleted HuCCT-1 cells the ability of CXCL12 to induce cell migration was reduced. Knock-down of beta arrestin-2 also reduced the ability of CXCL12 to induce cell migration. In contrast, pretreatment with pertussis toxin, a Gi inhibitor, affected CXCL12-induced cell survival but not CXCL12-mediated chemotaxis.

Conclusions: CCA cells express CXCR7 in vivo and in vitro. Activation of CXCR7 is involved in CXCL12-induced CCA migration and survival via activation of different signaling pathways.

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C-REL AND P27KIP ARE DOWNSTREAM TARGETS OF ERKS IN HEPATOCELLULAR CARCINOMA (HCC) IN VIVO AND IN VITRO

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Background/Aims: The molecular mechanisms underlying the development and progression of HCC remain poorly understood. The MAP kinase ERK5 has been implicated in tumor development. The goal of this study was to evaluate the relevance of this pathway ERK5 in HCC biology and to establish its downstream targets.

Methods: In Huh-7 and HepG2, ERK5 was silenced by siRNA transfection or with lentiviral vectors encoding specific shRNA. The
specific ERK5 inhibitor XMD8-92 was also used. In vivo development of HCC was evaluated using a xenograft model with Huh-7 in nude mice.

Results: In vitro, ERK5 silencing or inhibition caused growth arrest of HCC cells, affecting the G1/S transition. This phenotype was associated with an increased expression of p27Kip, a negative regulator of cell cycle progression. Upon ERK5 knockdown, expression of c-Rel, a member of the NF-κB family required for hepatocyte proliferation, was markedly downregulated. In a mouse model of HCC xenograft, ERK5 silencing or administration of XMD8-92 significantly decreased tumor volume. These effects were associated with reduced cell proliferation, as indicated by lower BrdU incorporation. Upon reduction of ERK5 activity in vivo, levels of c-Rel and of c-Jun, a proto-oncogene essential for cell proliferation, were reduced, while p27Kip was up-regulated. Expression of MEF2, a known target in the ERK5 pathway, was also inhibited.

Conclusions: We found that ERK5 regulates cell proliferation in HCC in vivo and vitro, affecting the expression of different oncogenic targets, including c-Rel, which we identify for the first time as a target of ERK5.

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T-48

POLYCOMB PROTEIN ENHANCER OF ZESTE HOMOLOG 2 (EZH2) PROTECTS HUMAN HEPATOCELLULAR CARCINOMA (HCC) CELLS FROM DNA DAMAGE RESPONSE (DDR) ASSOCIATED APOPTOSIS

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Background and aims: Chronic inflammation and double strand DNA breaks (DSBs) accumulation are associated during HCC development and progression with epigenetic modifications, including the recruitment at DSBs of the EZH2 histone methyltransferase. EZH2 is frequently overexpressed in HCC and correlate with aggressiveness and/or poor prognosis. Since EZH2 role in the modulation of DDR in HCC cells is largely unknown we investigated the modulation of EZH2 in response to DNA damage and the effects of EZH2 depletion on DDR associates apoptosis.

Methods: The human HCC cell lines HepG2, Huh6, Huh7, Hep3B and non transformed proliferating and differentiated HepaRG cells were exposed to doxorubicin 0.5 μM (low DNA damage/cell cycle arrest dose) or 2 μM (high DNA damage/apoptotic dose) for 24 h or 48 h. EZH2 was knocked down in HepG2 cells using lenti-viral delivery of shRNAs targeting EZH2.

Results: EZH2 knockdown significantly reduced HCC cell viability, which was recovered by overexpression of EZH2. EZH2 knockdown in HepG2 cells treated with doxorubicin 2μM resulted in a strong increase of DDR associates apoptosis.

Conclusions: EZH2 levels are modulated by DNA damage suggesting a potential role of EZH2 in DDR-associated cell cycle arrest and apoptosis in HCC cells. EZH2 activation contributes to HCC resistance to chemotherapeutic drugs further supporting the use of EZH2 inhibitors for HCC treatment.

http://dx.doi.org/10.1016/j.dld.2014.01.088

T-49

ANTAGONISTIC SIGNALLING BY PPARGAMMA AND ROSIGLITAZONE CONVERGE ON RUVBL1 REGULATION IN HCC CELLS

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Introduction: Ruvbl1, an AAA+ helicase involved in cell growth and oncogenic transformation, is overexpressed in several human cancers including HCC. We previously identified Ruvbl1 as a gene downregulated by Rosiglitazone (RGZ) in a PPARG-cKO mice prone to HCC.

Aim: To investigate the RGZ–PPARg axis in the regulation of Ruvbl1 and its biological relevance to HCC cell growth.

Materials and methods: Cell lines: Hepa1-6 and HepG2. Ruvbl1 expression was evaluated by qPCR, WB and IHC. Ruvbl1 promoter analysis was performed by CHIP and reporter assays. mRNA stability was evaluated by 3′-UTR reporter assay. HCC cell growth in vivo was evaluated through an orthotopic mice model.

Results: RGZ reduces Ruvbl1 expression in HCC cell lines. Surprisingly, PPARg silencing reduces both Ruvbl1 expression and promoter activity while its overexpression induces Ruvbl1 promoter activity, mRNA and protein. We localized functional PPREs in two proximal promoter regions. PPARg binding on Ruvbl1 promoter was confirmed by CHIP.

RGZ had negligible effects on Ruvbl1-1 promoter activity or its mRNA stability. Analysis of Ruvbl1 pre-mRNA suggests that RGZ impairs Ruvbl1 mRNA maturation.

Both PPARg and Ruvbl1 silencing impair HCC cell growth in vitro and reduce tumor formation in the orthotopic model. PPARg down-regulation reduced HCC cell viability, which was recovered by overexpression of Ruvbl1.

Conclusions: RGZ and PPARγ antagonistic each other in the regulation of Ruvbl1 expression. Knockdown of Ruvbl1 or PPARγ inhibitor reduces HCC cell growth in vitro and in vivo. Since Ruvbl1 regulates several cellular processes crucial for cancer, the net outcome of PPARg and RGZ antagonism may potentially elicit either pro- or anti-cancer effects.

http://dx.doi.org/10.1016/j.dld.2014.01.089
Introduction: Both ischemia–reperfusion injury (IRI) at operation and clinically assessed early allograft dysfunction during the first post-operative week (EAD) affect graft survival after liver transplantation (LT).

Aim: The aim of our study was to evaluate the impact of IRI and EAD on graft loss, according to recipient HCV status.

Materials and methods: We retrospectively analyzed 128 cirrhotic patients consecutively submitted to primary deceased-donor LT at our Center (November 2003–July 2011), with protocol coupled liver graft pre-ischemia and 1 h post-reperfusion biopsies. Several recipient, donor and graft variables, including pre-ischemia steatosis, were prospectively collected. IRI was categorized as mild-moderate or severe.

Results: Median recipient and donor age were 54 (18–71) and 48 (9–81) years, respectively. Median graft follow-up was 15 months (6–180). Median AST and ALT peak during the first post-operative week were (9–81) years, respectively. Median graft follow-up was 47 months, 95% CI 1.072–8.289; p = 0.885) recipients; (b) the occurrence of EAD independently predicted graft loss in HCV− (HR 2.982, 95% CI 1.072–8.289; p = 0.036), but not in HCV+ (HR 1.516, 95% CI 0.589–3.900; p = 0.388) recipients.

Conclusions: IRI but not EAD predicts graft loss in HCV+ recipients, while the opposite is true for HCV− recipients. Post-reperfusion biopsy should be performed in HCV+ recipients.

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T-51

LIVER TRANSPLANTATION FOR COMBINED HEPATOCELLULAR CARCINOMA AND CHOLANGIOCARCINOMA: A CASE MATCH ANALYSIS

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Introduction: Combined hepatocellular carcinoma and cholangiocarcinoma (chCC–CC) is a rare tumor of difficult differential diagnosis with hepatocellular carcinoma (HCC) or cholangiocarcinoma (CC). Therefore some patients who undergo liver transplantation (LT) for a supposed HCC are eventually diagnosed with chCC-CC.

Aim: The aim of this study is to evaluate incidence, characteristics, preoperative treatments and post transplant outcomes of patients who underwent LT for HCC and were found to have chCC-CC at final pathology.

Materials and methods: Since 1991–2012 we retrospectively analyzed all patients who underwent LT for chCC-CC in our Center. Pathologic specimens were re-analyzed to confirm the diagnosis. Demographics, clinical data, survival and outcomes after LT were compared to patients with HCC. Furthermore we performed a case match analysis pairing patients 1:2 (chCC-CC:HCC) by age, sex, and the following pre-LT tumor characteristics: number of nodules, maximum size of biggest nodule and sum of total tumors diameter.

Results: During the study period 24 patients underwent LT for chCC-CC and 299 for HCC. Patients affected by chCC-CC were younger (55.9 vs 60.3 years; p = 0.01). No differences were found in preoperative patient and tumor characteristics. Patients affected by HCC received significantly more pre-LT treatments (p = 0.03), particularly alcohol injection (p = 0.02). No differences in recurrence were found (chCC-CC 22.2% vs HCC 20.4%). Survival at 1 and 3 years were significantly different between groups, (85% and 75% for HCC vs 67% and 60% for chCC-CC; p = 0.04). When chCC-CC and HCC were paired with a ratio 1:2 no differences in overall survival were observed.

Conclusion: In our experience chCC-CC and HCC had a similar recurrence rate and a similar oncologic outcome according to the case match analysis. Preoperative diagnosis of chCC-CC is often not possible and no clinical protocols are described regarding best immunosuppression regimen or adjuvant treatments. Multicenter studies will be necessary.

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T-52

OPTIMIZING RESULTS OF LIVER TRANSPLANTATION WITH GRAFT FROM HBcAb POSITIVE DONOR

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Introduction: In recent studies, the outcome of liver transplantation (LT) with graft from HBcAb-positive donor (D\(\text{HBcAb-Pos}\)) appeared to be markedly influenced by the etiology of liver disease in the recipient.

Aim: To evaluate the survival of D\(\text{HBcAb-Pos}\) grafts and the results of the change of their allocation in our Center which occurred 3 years ago.

Materials and methods: From 11/2002 to 12/2012, we performed 1148 first LTs in adult recipients, 205 (18%) of which with D\(\text{HBcAb-Pos}\). The whole period was split in two eras: Period\(_1\), 11/2002-08/2010, 878 LTs with liberal allocation of 160 D\(\text{HBcAb-Pos}\) grafts; Period\(_2\), 09/2010-12/2012, 270 LTs with allocation of 45 D\(\text{HBcAb-Pos}\) grafts only to HCV-negative recipients.

Results: In Period\(_1\), 72 (45%) D\(\text{HBcAb-Pos}\) grafts were allocated to HBV recipients, 59 (37%) to HCV recipients, 29 (18%) to recipients with Other Indications. At 36 months, graft survival related to recipient’s etiology was: HBV 93.4% with D\(\text{HBcAb-Neg}\), 87.5% with D\(\text{HBcAb-Pos}\); HCV 73.8% with D\(\text{HBcAb-Neg}\), 54.2% with D\(\text{HBcAb-Pos}\); other indications 86.5% with D\(\text{HBcAb-Neg}\), 82.8% with D\(\text{HBcAb-Pos}\). At 60 months: HBV 92.5% with D\(\text{HBcAb-Neg}\), 86.1% with D\(\text{HBcAb-Pos}\); HCV 65.3% with D\(\text{HBcAb-Neg}\), 43.0% with D\(\text{HBcAb-Pos}\) \(\text{p}=0.002\); Other Indications 82.1% with D\(\text{HBcAb-Neg}\), 72.4% with D\(\text{HBcAb-Pos}\). At Cox-regression in Period\(_1\), risk factors for graft survival were: D\(\text{HBcAb-Pos}\) grafts; Period\(_2\), 09/2010-12/2012, 270 LTs with allocation of 45 D\(\text{HBcAb-Pos}\) grafts only to HCV-negative recipients.

Conclusions: The change in the allocation of D\(\text{HBcAb-Pos}\) grafts in Period\(_2\) was justified by the unsustained results obtained in HCV recipients in Period\(_1\) (survival less than 50% at 5 years), and enabled to optimize results of LT with D\(\text{HBcAb-Pos}\) grafts in the medium term.

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T-53

LIVER TRANSPLANTATION IN CIRRHOTIC PATIENTS WITH PORTAL VEIN THROMBOSIS: A SINGLE CENTRE EXPERIENCE

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Introduction: Portal vein thrombosis (PVT) is a complication of cirrhosis that may increase surgical complexities during orthotopic liver transplantation (OLT) and cause complications after surgery.

Aim: To evaluate the management of PVT before, during and after OLT in our centre.

Materials and methods: Among all the cirrhotic patients who underwent OLT between 2005 and 2011 in Turin Liver Transplantation Centre, we retrospectively included all the patients with US and CT diagnosis of pre-OLT non-neoplastic PVT. Extension of thrombosis (according to Yerdel classification), presence of genetic prothrombotic risk factors, pre-OLT clinical and US course, use of anticoagulation therapy (AT), surgical technique for portal vein anastomosis, complications and US follow-up after OLT were collected for each patient.

Results: 70/997 (7%) patients were included. PVT was: intrahepatic in 22.9% of them, grade 1 in 32.9%, grade 2 in 24.3%, grade 3 in 7.1% and grade 4 in 12.8%.

We found very small prevalence of genetic prothrombotic risk factors, and their presence did not correlate with extension of thrombosis and US course.

Due to thrombosis, 72% of patients started AT (complications rate: 17%, all minor bleedings) and 40% underwent TIPS, without complications. Pre-OLT complete resolution or regression of thrombosis occurred in 74% of patients under AT vs 40% of patients not treated \(\text{p}=0.04\). During OLT 97% of patients underwent porto-portal anastomosis, 29% of them needing thrombectomy. PVT extension (both at diagnosis and at OLT) and use of AT did not statistically impact in terms of survival and complications during and after OLT.

Conclusions: PVT is a frequent issue in cirrhotic patients waiting for OLT and its development seems to be unrelated to the presence of prothrombotic risk factors. In our experience AT can be safely managed allowing a pre-OLT understaging of PVT and the need for special surgical techniques at OLT is very uncommon.

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**T-54**
RECIPIENT AND DONOR GENDER MATCH PREDICTS THE OUTCOME OF ANTIVIRAL THERAPY FOR RECURRENT HEPATITIS C

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**Background and aim:** The aim of the study is to evaluate whether donor gender has an impact on SVR and if there could be an interaction between recipient and donor gender in determining the outcome of antiviral therapy for recurrent hepatitis C (RHVC).

**Methods:** The study included 397 (299 males, median age 55 years) HCV positive recipients (273 HCV G1, 61 G2, 37 G3, 17 G4) with RHVC, from 12 Italian liver transplant centers, treated for 48 weeks with interferon-α and ribavirin. Median donor age was 49 years and 245 (61.7%) were males. All patients were treated for 48 weeks with interferon-α and ribavirin.

**Results:** SVR was achieved in 139/397 (35.0%) patients. The strongest predictors of SVR at univariate analysis were: presence of early viral response (EVR), donor age, adherence >80% of IFN schedule, HCV genotypes 2/3, basal viral load, recipient male gender and use of pegylated IFN. Patients were divided in four groups according to the donor (D) and recipient (R) gender match (M = male, F = female). A significant association between donor and recipient gendermatch and the overall SVR was observed: RF/DF 12/54 (22.2%) vs RF/DM 13/44 (29.5%) vs RM/DF 34/102 (33.3%) vs RM/DM 80/197 (40.6%) (p = 0.007). In HCV G1/4 infected patients SVR was observed in: 6/46 (13.0%) RF/DF vs 7/36 (19.4%) RF/DM vs 20/79 (25.3%) RM/DF vs 39/138 (28.3%) RM/DM (p = 0.029). Stepwise logistic regression analysis confirmed recipient/donor gender mismatch as independent predictor of SVR (OR 0.739, 95% C.I. 0.5618–0.9731, p = 0.0312).

**Conclusions:** Female gender in both recipient and donor play a negative role in terms of response to antiviral therapy for RHVC.

http://dx.doi.org/10.1016/j.dld.2014.01.094

**T-55**
THE IMPACT OF IL-28B POLYMORPHISM AND DIABETES ON SVR AFTER ANTIVIRAL THERAPY (AT) FOR POST-LIVER TRANSPLANT (LT) HCV RECURRENCE

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6 Gastroenterology, University of Padua, Italy
7 Gastroenterology, University of Bari, Italy
8 Gastroenterology, A. Gemelli Hospital, Rome, Italy
9 San Camillo Spallanzani Hospital, Rome, Italy
10 University of Torvergata, Rome, Italy
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13 Medical Liver Transplant Unit, Department of Medical Sciences Clinical and Experimental, University of Udine, Italy

**Background:** HCV recurrence after LT is a major cause of reduced survival. Diabetes and insulin-resistance are well known negative prognostic factors for SVR to Dual AT in the non-transplant setting. Donor/recipient IL-28B polymorphisms can be a predictor of SVR. This study aimed at investigating the interactions between calcineurin inhibitors, IL-28B polymorphisms, diabetes and SVR after LT.

**Methods:** This retrospective multicenter study included 381 LT patients (LTR) treated with dual AT for recurrent HCV from 2001 to 2009. In a subgroup of 147 patients IL-28B rs12979860C/T polymorphism was assessed in both donors and recipients.

**Results:** A diagnosis of diabetes mellitus was made in 148/381 (38.3%) LTR. The only factor found to be associated to DM was recipient/donor IL-28B genotype in patients under tacrolimus: 4C-alleles 0/7 (0.0%), 3C-alleles 8/31 (25.8%) and ≤2C-alleles 18/41 (43.9%), p = 0.012 for linear trend. Overall SVR rate was 35.4% (135/381): 12/14 (85.7%) G1–3 vs 60/144 (41.4%) G2–4. A diagnosis of diabetes was associated with lower rates of SVR in the whole cohort (39/146 vs 96/235, p = 0.007) and in G2–3 patients (11/27 vs 49/59, p = 0.0002), but not in G1–4 alone. The association between diabetes and SVR was confirmed by logistic regression in G2–3 patients. Analysis of risk factors for no response to dual AT: according to DM, tacrolimus and carrying less than 3 IL-28B “C” alleles, patients were stratified in four groups: (a) no risk factors; (b) 1 risk factor; (c) 2 risk factors; (d) 3 risk factors with the following rates of SVR: (a) 19/29 (65.5%); (b) 22/64 (34.4%); (c) 13/36 (36.1%); (d) 1/18 (5.6%), p = 0.0002 for linear trend.

**Conclusions:** Donor and recipient rs12979860 alleles influence the onset of diabetes in tacrolimus-treated group, and the diabetes itself has a significant impact on the attempt of sustained virological response rate to AT for recurrent hepatitis C.

http://dx.doi.org/10.1016/j.dld.2014.01.095
OXYGEN GRADIENT ON A CHIP FOR OPTIMIZING THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO HEPATIC CELLS

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Developing new strategies for deriving mature functional hepatocytes from human pluripotent cells have a high therapeutic potential and could be used to study molecular and genetic aspects of human hepatic disease and development. Nowadays the role of oxygen tension in hepatic differentiation is not well described.

Aims: (a) To recapitulate early in vitro organogenesis in physiological conditions to efficiently derive mature hepatic cells from hPSCs and hESCs under a stable oxygen gradient. (b) To integrate the specific lineages into a microfluidic platform to obtain a functional liver tissue on a chip.

Materials and methods: Our multi-stage microfluidic technology allows us to differentiate human induced pluripotent stem cells (cell line ADHF#1, iCEMS, Kyoto University) and human embryonic stem cells (cell line HES2, National Stem Cell Bank, Madison WI) into mature cells. Microfluidic technology is also implemented with 1 mm oxygen gradient generation to obtain hepatocyte-like cells which express hepatic markers (alpha-fetoprotein, cytokeratins 18, 19, albumin, CYP3A) and have functional competences (glycogen storage, indocyanine green uptake, albumin secretion).

Results: We developed a microfluidic technology that generates a stable Oxygen gradient and allows to culture and differentiate human pluripotent cells (hPSCs and hESCs) into hepatocyte-like cells. The cells obtained show an oxygen-gradient dependent AFP and CYP3A expression, glycogen storage and albumin secretion over a 10-day (AFP) and 14-day period (CYP3A, Albumin). Our microfluidic technology allowed to accurately control human pluripotent cells expansion and fate toward endoderm commitment, hepatic development and functional maturation on a chip.

Conclusions: The engineerization of pluripotent cell differentiation into hepatic lineages under defined oxygen gradient will allow us to further understand the mechanisms involved in tissue development. Moreover, mature hepatic cells fully integrated on a chip could be directly used for temporal-defined toxicological assays and drug screening.

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THE PREDICTIVE ROLE OF WEEK 8 (RVRW8) VIRAL KINETICS IN PRIOR PR NULL RESPONDERS RECEIVING TELAPREVIR IN THE DELAYED-START ARM OF THE REALIZE TRIAL


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Introduction: The approved label for telaprevir-based treatment does not recommend a delayed start (DS), although in clinical practice it may be used to evaluate response to peginterferon-alfa and ribavirin (PR). The REALIZE trial evaluated the addition of telaprevir to PR (w/o 4-week period during which patients received PR only) in treatment-experienced patients infected with hepatitis C virus (HCV).

Aim: The aim of these analyses was to clarify the role of rapid virologic response (RVR) at Week 8 (HCV RNA undetectable after 4 weeks of PR and 4 weeks of telaprevir/PR) in identifying prior null responders who will achieve an SVR with telaprevir-based triple therapy.

Materials and methods: REALIZE was a randomised, placebo-controlled, Phase III trial evaluating telaprevir plus PR in HCV-1-patients with prior relapse (n = 354), prior partial response (n = 124) or prior null response (n = 184). Patients received Peg-IFN (180 μg/week), RBV (1000/1200 mg/day), TVR (750 mg q8h) for 12 weeks plus either 32 (DS) or 36 (no DS) weeks of PR alone. 264 patients (141 prior relapsers, 48 prior partial responders, 75 prior null responders, 125 with bridging fibrosis/cirrhosis) were enrolled in the DS arm.

Results: RVR at Week 8 was the best predictor of SVR in all prior response groups (overall SVR in RVRw8+: 82%). More than two thirds of all prior null responders achieving an RVRw8 subsequently achieved an SVR (68%) compared with only 9% of patients not achieving an RVRw8 (p = 0.0001) (Table 1).

Conclusions: In the DS arm of REALIZE, RVR at Week 8 allows identification of prior null responders who are most likely to achieve an SVR.

Table 1

<table>
<thead>
<tr>
<th>RVRw8+</th>
<th>T12(DS)/PR48 n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All prior null responders</td>
<td>31/75 (41)</td>
</tr>
<tr>
<td>F3/F4</td>
<td>15/45 (33)</td>
</tr>
<tr>
<td>SVR</td>
<td>25/75 (33)</td>
</tr>
<tr>
<td>All prior null responders</td>
<td>8/31 (26)</td>
</tr>
<tr>
<td>F3/F4</td>
<td>7/15 (47)</td>
</tr>
<tr>
<td>SVR in RVRw8+</td>
<td>21/31 (68)</td>
</tr>
<tr>
<td>All prior null responders</td>
<td>4/43 (9)</td>
</tr>
<tr>
<td>F3/F4</td>
<td>3/30 (10)</td>
</tr>
</tbody>
</table>

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F-02

HCV GENOTYPE 1A AND 1B: SIMILARITIES AND DIFFERENCES IN CLINICAL FEATURES, THERAPEUTIC OUTCOME AND PREDICTORS OF RESPONSE

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Introduction and aim: The majority of clinical trials assessed treatment response rate among HCV genotype 1 patients as a single group and it has been unable to uncover differences in treatment outcome between the two subtypes. We aimed to evaluate the baseline similarities and differences between the two HCV-1 subtypes, 1a and 1b, on pre-treatment and on-treatment predictors of response to peg-interferon (PEG-IFN) and ribavirin.

Methods: 1184 naïve patients with HCV genotype-1 infection were treated with PEG-IFN in combination with daily ribavirin (1000–1200 mg/day). Between January 2005 and December 2010 a total of 15 centers in Italy, selected by voluntary participation, took part into the study. The study included 155 (13%) patients infected with subtype 1a and 1029 (87%) patients with subtype 1b.

Results: Six factors differentiate genotype 1a vs. genotype 1b: more frequent male sex (74% vs. 56%; p < 0.001); age younger than 50 years (63% vs. 30%; p < 0.001); value of BMI (24 vs. 25; p < 0.042); higher value of platelet count (209 x 103 vs. 186 x 103; p < 0.001); less frequent severe liver fibrosis (70% vs. 52%; p < 0.001) and finally less frequent type 2 diabetes comorbidity (5% vs. 12%; p < 0.005). All other parameters resulted equally distributed between the two HCV 1 subtypes. Of note, the IL28B polymorphisms and the RVR resulted equally distributed between the two HCV 1 subtype. SVR is achieved by 38% of genotype 1b and by 45% of genotype 1a even in this difference of 7% is not statistically significant (p = 0.076).

Conclusions: The study, conducted in a large nation-wide cohort of naïve patients with HCV subtype 1a and 1b infections, shows that genotype 1a is more frequently observed in young male patients. The study also pinpoints some differential predictive features of SVR between the two subtypes, a finding which would imply that the two subtypes must be separately evaluated in future therapeutic trials.

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F-03

EARLY VIRAL DYNAMICS IN HCV-RNA DECAY AND NS3-RESISTANCE DEVELOPMENT PREDICT THE RISK OF FAILURE TO FIRST-GENERATION PROTEASE INHIBITORS


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Introduction: Aim of this study was to evaluate, in clinical practice, HCV-RNA kinetics and baseline/early NS3-protease resistance-associated-variants (RAVs) during triple-therapy with telaprevir/boceprevir + peg-IFN + RBV, in order to identify patients with high-risk of virological failure.

Methodology: 126 patients (GT1a/1b/1g = 46/79/1; previously non-responders/relapsers/naive = 75/31/20; cirrhotic - 61/48/4%) received peg-IFN + RBV + telaprevir (N = 89) or + boceprevir (N = 37). Presence of RAVs (36AGLM-1IR-43ISV-54AVP-55A-80K-155KMQT-156GSTV-170AT-174F) was evaluated by population-sequencing at baseline, by early time-points (48h-2weeks) and at failure. HCV-RNA was determined at baseline/early-time points and then following treatment-protocol.

Results: In this ad interim analysis, virological-failure was observed in 32 (25%) patients (72% previously non-responders, 93.3% CT/TT), in the 88% of cases associated with RAVs. Both baseline-RAVs (9/112 patients) and early-RAVs (7/29 patients) were detected (V36M-T54AS-Q80K-R155K-T156TV). Among patients with available treatment-outcome (N = 67), baseline/early presence of RAVs was predictive of failure (OR[CI] = 4.118[1.216–13.946], p = 0.023), differently from the detection of RAVs exclusively at baseline (OR[CI] = 2.857[0.619–13.184], p = 0.178). In particular, 10/15 (66.7%) patients with baseline/early RAVs failed treatment vs. 17/52 (32.7%) of patients without (p = 0.032). In the case of GT1a previous non-responders, 2/2 patients with baseline boceprevir/telaprevir-RAVs (1 = V36I; 1 = T54S) and 4/4 patients with Q80K simpeprevir-RAV failed treatment. Differently, 2/2 GT1b patients (previous relapsers) with baseline-RAVs (T54S; Q80K) are SVR2s.

Regarding early HCV-RNA kinetics: the median [IQR] decay of HCV-RNA after 48 h of telaprevir-administration was substantial (−3.0[−3.4]: −2.6[logUI/ml] and independent from failure to triple-therapy (p = 0.941). Contrariwise, week-2 HCV-RNA decay was significantly lower in failing-patients than in those who reached End-Of-Treatment (−3.7[−4.3]: −3.2) vs. −4.6[−5.2]: −4.0[logUI/ml], p = 0.007). None of the 11 patients with undetectable HCV-RNA at week-2 subsequently failed telaprevir-treatment, vs. 2/27 patients with detectable HCV-RNA < 100 IU/ml, and 10/18 patients with HCV-RNA > 100 IU/ml (p = 0.001). Similarly, 6/6 patients with HCV-RNA > 100 IU/ml at week-4 of boceprevir-administration subsequently failed, vs. 2/13 patients with HCV-RNA.

Conclusions: Among PI-regimens, especially for difficult-to-treat patients, early-detection of RAVs (including Q80K), combined with a suboptimal HCV-RNA decay in the first weeks of triple-therapy, may identify patients with higher risk of virologic-failure, thus requiring a closer clinical-monitoring.

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F-04

ELIGIBILITY 2: A PROSPECTIVE, OBSERVATIONAL, MULTICENTRE STUDY ON OUTCOMES OF PEG-INTERFERON/RIBAVIRIN (PEG-IFN/RBV) IN CHRONIC HEPATITIS C (CHC)

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Background and aims: To identify the reasons of discontinuation of Peg-IFN/RBV and characteristics of treatment failure of CHC patients in real Italian clinical practice.

Methods: 1.128 CHC patients referred to 45 hepatology centres were enrolled in a phase 4, prospective, observational study. Prescription of Peg-IFNa2a or a2b was at discretion of hepatologist; difference in response between the two drugs was not a study objective.

Results: 687/1,118 subjects (61.4%) were eligible to antiviral treatment. 598 (87.0%) agreed doctor’s decision. Outcome information was available in 500/598. Of 500 treated, 348 (69.6%) completed the treatment. 152 discontinued treatment for: lack of response (28.9%), personal reasons (29.6%), adverse events (AEs, 38.2%), uncompensated hepatic decompensation (1.3%). 263/500 (52.6%) obtained a sustained virological response (SVR), 71 (14.2%) relapsed and 61 (12.2%) were non-responders. Treatment outcome information was not available in 105/500 (21%) since: lost on-treatment (33.3%),
lost during follow-up (25.7%), withdrawn for AEs (19.1%), administrative reasons (21.9%). Based on HCV genotype, intention-to-treat (n = 500) and per-protocol (n = 395) analyses of SVR rates are shown in Table 1. The safety evaluation is not applicable to this study since it is a phase 4, sponsored study and the reported AEs concerned only MSD products.

Conclusions: In Italian clinical practice only 61% of HCV+ patients are considered eligible to PegIFN/RBV. Among these, 13% refuse treatment proposal. About 30% do not complete the scheduled treatment and despite this, SVR rate is similar to that of randomized-controlled trials. These data offer important implications in the era of triple antiviral therapy, both for assessing eligibility and estimating drop-outs rates.


http://dx.doi.org/10.1016/j.dld.2014.01.102

F-05

EARLY PREDICTION OF SUSTAINED Virologic RESPONSE BY HCV Core AG KINETICS IN CHRONIC HEPATITIS C PATIENTS TREATED WITH PegIFN + RBV

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Introduction: Monitoring of viral load decline to assess the antiviral efficacy of treatment is mandatory to warrant an appropriate management of chronic hepatitis C patients (CHC pts). Testing of serum HCV Core Antigen (HCVcAg) represents an alternative tool to monitor the dynamics of viral infection.

Aim: To study the correlation between the kinetics of HCVcAg and HCV-RNA decline and their diagnostic performance for prediction of sustained virological response (SVR).

Materials and methods: 125 genotype 1 CHC pts (78.4% males: 78.4% 1b; 62.4% naïve; 59.2% F3–F4), treated with Peginterferon–Ribavirin (P/R) were tested at 0–2–4–7–14–21–28–84 days during therapy for HCV-RNA (Cobas, Roche) and HCVAg (ARCHITECT, Abbott).

Results: HCVAg and HCV-RNA serum levels strongly correlated at baseline and during therapy (p < 0.001). Their baseline levels were not influenced by sex, age, subtype, IL28B, basal ALT and steatosis. Overall 54 (43.2%) pts had SVR, 25 (20%) of them with a rapid virologic response (RVR: HCV-RNA < 25 IU/mL at day 28).

The diagnostic performances in predicting SVR of RVR and of undetectable HCVcAg at different time points are reported in the table.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Other or not determined</td>
<td>All</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>52.4%</td>
<td>98.1%</td>
<td>98.1%</td>
<td>98.1%</td>
<td>96.3%</td>
</tr>
</tbody>
</table>

Conclusions: The kinetics of serum HCVcAg decline during P/R treatment parallels that of HCV-RNA in genotype 1 CHC pts. The high specificity of HCVcAg in predicting SVR as early as 1 week after treatment start and the high probability (91.3%) of SVR in pts with undetectable HCVcAg levels at day 21 suggests HCVcAg as a suitable, cheap and rapid diagnostic tool to tailor antiviral treatment.

http://dx.doi.org/10.1016/j.dld.2014.01.103

F-06

ELIGIBILITY 1: A PROSPECTIVE STUDY EVALUATING HCV PATIENTS’ CHARACTERISTICS OF ELIGIBILITY FOR ANTIVIRAL THERAPY IN REAL CLINICAL PRACTICE

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Introduction: Chronic HCV infection is the leading cause of mortality from liver cirrhosis and hepatocellular carcinoma (HCC). Pegylated interferon (Peg-IFN) and ribavirin (RBV) can prevent the disease progression in chronic hepatitis C (CHC).

Aim: This prospective multicenter study investigated treatment eligibility for Peg-IFN + RBV in CHC in the Italian clinical practice.

Materials and methods: A total of 1,128 CHC patients referred to 45 centers were prospectively enrolled in the study. HCV-RNA-negative and previously treated patients were excluded. Non-eligibility criteria were analyzed. 1,118 were included in the analysis. There was a slight prevalence of males (51.6%), the mean age (±SD) was 55.5 ± 14.5. The mean body mass index was 25.2 ± 4.3 with no gender differences. The most represented age decade was 60–69 years (23.8%). The diagnosis was CHC without cirrhosis in 981/1118 (87.7%), compensated cirrhosis in 106/1118 (9.5%), decompensated cirrhosis in 24/1118 (2.1%) and HCC in 7/1118 (0.6%). Only 44/1118 (3.9%) were above limit alcohol consumers. Genotype 1 was the most represented (47.3%) followed by 2 (23.8%), 3 (13.1%) and others (15.8%). The most frequent concomitant diseases were vascular (21.4%), metabolic (12.2%) and psychiatric (10.8%). 431/1118 (38.6%) was judged as non-eligible for antiviral therapy. Reasons of non-eligibility are analyzed in the table.

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Conclusions: In Italian clinical practice, almost 40% of patients with CHC are considered non-eligible for treatment. Patient's age and comorbidity represent the most important reasons of non-eligibility. Considering substantially shorter treatment durations with the addition of direct-acting antivirals, eligibility can be increased with careful evaluation of risk/benefit profile for individual patients along with efficient management of comorbidities.

Grant Support: Study sponsor for drug supply and financial support: Schering-Plough (now Merck) SpA, Milan, Italy.

ClinicalTrials.gov <http://ClinicalTrials.gov> Identifier: NCT00724451.

Main reasons of eligibility

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<tr>
<td>Age</td>
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<td>Histologically mild chronic hepatitis</td>
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<td>17.9%</td>
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<td>Substance abuse</td>
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<tr>
<td>Contraindicated:</td>
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<td>Comorbidity</td>
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<td>Severe hepatic dysfunction</td>
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http://dx.doi.org/10.1016/j.dld.2014.01.104

F-07

DOES ULTRA-DEEP-SEQUENCING IN HCV PATIENTS TREATED WITH BOCEPREVIR/TELAPREVIR-BASED THERAPY PROVIDE AN ADDED VALUE IN COMPARISON TO STANDARD POPULATION-SEQUENCING IN THE DETECTION OF RESISTANCE?

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Background: Aim of this study was to compare in clinical practice ultra-deep-454-pyrosequencing (UDPS) versus population-sequencing on the detection of NS3-protease resistance-associated-variants (RAVs) at baseline/during/after triple-therapy with telaprevir/boceprevir + pegIFN/RBV.

Methods: NS3-protease sequences of 38 selected patients (HCV-1a/1b = 16/22); previous-non-responders/relapsers/naive = 26/10/2; cirrhotic = 22) treated with pegIFN/RBV + telaprevir (N = 32) or +boceprevir (N = 6) (20-virological-failures; 18-responders) were analyzed. Presence of RAVs (36AGLM-41R-43ISV-54ASV-55A-80K-155IKMQT-156GSTV-170AT-174F) was evaluated by both population-sequencing and UDPS (cutoff = 0.1%; >3000sequences/patient) at baseline, early-time-points (8–12–24–48 h), failure, after therapy-interruption.

Results: At baseline, UDPS confirmed the population-sequencing detecting resistance in 10/38 patients. Additional baseline-minority-RAVs were found in 3 telaprevir-treated patients only by UDPS. In particular, two HCV-1a previous-null-responders with baseline-minority-RAVs (pt1:F43S:0.13%; pt2:Q80K:0.6%) failed telaprevir-triple-therapy with V36M + R155K and V36A + R155K + T156ST, at 22- and 2-weeks,
respective. The other HCV-1b patient, previous-relaper, with baseline-minority-RAV T54A (0.28%) achieved SVR24.

In 19 patients analyzed at early-time-points, UDPS confirmed the population-sequencing results, detecting resistance in 6/19 patients. By UDPS, additional early-minority-RAVs were detected in 3 patients without baseline-resistance (pt1:V36A48h = 2.5% [=183 IU/ml] + R155 K48h = 4.1% [=301 IU/ml]), and then failed with a similar RAV-pattern (V36M + R155K = 100%).

Finaly, to characterize also the persistence of RAVs, 6 failing-patients were analyzed by both UDPS and population-sequencing after therapy-interruption (median[IQR] time:18[10-26] weeks).

3/6 patients (2 = HCV-1a; 1 = HCV-1b) showed RAVs by both methods at 11/34/48-weeks after therapy-interruption, respectively. 2 patients (HCV-1b) showed persistence of minority-RAVs only by UDPS (T54S = 16.6%; V36A = 0.68%), at 12/32-weeks, respectively. The last patient (HCV-1b) showed no longer resistance found previously at failure (V36A + T54AS) by both methods at 12-weeks after therapy-interruption.

Conclusions: UDPS provides an added-value on resistance detection at all time-points analyzed. Although its clinical relevance is still poorly understood, it could be used for choosing the later soon-available treatment in patients who failed first-generation PIs.

http://dx.doi.org/10.1016/j.jdd.2014.01.105

F-08

HCV RNA VIRAL LOAD IS INDEPENDENT FROM CD4 CELL COUNT AND PLASMA HIV RNA VIRAL LOAD IN IMMUNOCOMPETENT HIV-HCV CO-INFECTED PATIENTS: A 3-YEARS FOLLOW-UP STUDY

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Introduction: HCV RNA viral load has a known role as predictor of sustained virological response and, recently, a significant correlation with liver fibrosis was described.

Aim: To investigate if the studied variables can influence HCV viral load in HIV-HCV co-infected patients over a study time of three years (2009–2012).

Materials and methods: Adult HIV-HCV co-infected subjects were enrolled if they had a detectable plasma HCV RNA in 2009 and 2012, a viro-immunological follow-up of HIV disease every 3 months, no severe comorbidities, if they were HBsAg negative, HCV therapy naïve or with antiviral treatment failed before June 2008. Statistical methods: linear regression analysis, ANOVA, Fisher’s exact test, t-test and mixed mode (ME) maximum likelihood linear regression model.

Results: 82 patients were included [M/F 62/20, mean age 48 years, 48 subjects with genotype 1 and 19 with advanced liver fibrosis]. Most patients were on antiretroviral therapy (89%), only a minority were nonresponders to a previous HCV treatment (20.7%). HCV RNA level did not change significantly from 2009 to 2012 (from 3924650 ± 5320177 IU/ml to 3085128 ± 3372247 IU/ml, P = 0.13; the CD4 count increased significantly (from a mean of 576 to a mean of 654, P = 0.003). Using linear regression a positive correlation with log10HCV RNA was found for genotype 1 (P < 0.001) and for being a nonresponder patient (P = 0.04): other studied covariates (age, gender, advanced liver fibrosis, ongoing HIV therapy, CD4 + cell count, HIV viremia detectable or undetectable) failed to reach a significant correlation.

Conclusions: HCV RNA value is higher inco-infected patients with genotype 1 and in previously nonresponders but it remains independent from the two main parameters of HIV disease, plasma HIV RNA and CD4 cell count, in an observation time of 36 months in patients with recovered or spontaneously maintained immunocompetence.

http://dx.doi.org/10.1016/j.jdd.2014.01.106

F-09

ASSOCIATION OF IFNL4 GENOTYPE AND ALT DECREASE IS A NEW TOOL TO PREDICT VIRAL ERADICATION IN HCV SARDINIAN PATIENTS WITH CHRONIC HEPATITIS C, GENOTYPE 4, TREATED WITH PEG-IFNα AND RIBAVIRIN

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Background and aim: HCV is a major cause of liver disease in the world. Sustained virological response with PEG-IFNα and Ribavirin is in about 60% of patients, according to genotype. Genotype 4 seems to have a higher response compared to genotype 1. In Sar- dinia HCV prevalence is high (about 5%) and genotype 4 is more representative than in the Italian mainland (15% vs. 3%). Two polymorphisms in the IFNL3 gene region (rs1297860 and rs8099917) are associated with SVR. Another new polymorphism in the IFNL4 gene region seems to be associated with response to IFN-α therapy. The aim was to determine the contribution of IFNLs polymorphisms, and other host and viral factors in predicting response to therapy among Sardinian patients infected chronically with HCV genotype 4.

Methods: We retrospectively analyzed 62 consecutive Sar- dini patients with HCV chronic hepatitis, mean age 41 ± 8.16 years, 80.6% males, 12 patients (19.3%) received IFN alfa in the past. 58 of 62 (93.5%) underwent to liver biopsy and 10 (17.2%) had fibrosis >F3 or cirrhosis. All patients were treated with PEG-interferon alfa (49 with 2a and 13 with 2b) and Ribavirin according to Standard of Care between 2003 and 2012 at the Liver Unit of University of Cagliari.
Results: 33 patients (53.2%) achieved SVR, 18 (29%) were non responders and 11 (17.8%) relapsed. There was no difference at the baseline regarding age, sex, BMI, previous antiviral therapy, HCV RNA, laboratory tests, histological score. IFNL4 genotype was identified as the most important factor associated with SVR, together with the decrease of ALT. Patients with ΔGss469415590 genotype and SVR presented flares of ALT in the first phase of therapy.

Conclusion: The combination data of IFN-λ polymorphisms and ALT reduction can give the opportunity to the clinicians to apply the best option among standard PEG-IFNα/Ribavirin and protease/polymerase inhibitors.

http://dx.doi.org/10.1016/j.dld.2014.01.107

F-10

FCγRIIIa SHEDDING FROM NK CELLS DOES NOT IMPAIR ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY IN CHRONIC HCV INFECTION

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Introduction: CD16 (FcγRIIIa) is a potent NK cell activating receptor that mediates Ab-dependent cell-mediated cytoxicity (ADCC). Reduced NK CD16 expression, leading to impairment of ADCC, has been linked to increased disease severity in chronic viral infections.

Aim: We evaluated CD16 expression on NK cells in patients with chronic HCV infection (HCVp) to understand regulatory mechanisms and their significance in ADCC efficiency.

Materials and methods results: NK cell surface CD16 expression and mRNA levels were examined in HCVp (n = 53) and healthy donors (HD, n=38) by flow cytometry and RT-PCR, respectively. To determine the effects of HCV on CD16 expression, PBMC and purified NK cells were exposed to culture-derived HCV (HCVcc)-infected Huh 7.5 cells before and after MMP inhibition or Galectin (Gal)-9 blockade, both of which are known to modulate CD16 on NK cells. ADCC was evaluated using trastuzumab-coated HER2+ target cells. Ex vivo NK cell CD16 expression and mRNA levels were lower in HCVp compared with HD (p<0.0001 and p = 0.018, respectively). Following exposure to HCVcc-infected cells, HD PBMC and purified NK cells significantly down-modulated CD16 (p<0.001) which was variably influenced by Gal-9 blockade and reproducibly, though not completely, restored by inhibition of MMPs, which regulate CD16 shedding. ADCC was increased in HCVp compared with HD independently of CD16 expression.

Conclusions: HCV down-modulates NK CD16 expression in vitro and in vivo predominantly, but not exclusively, via MMP-dependent shedding. Reduced NK CD16 expression had no negative effect on ADCC in HCVp suggesting that the described polarization of NK cells toward cytotoxicity overwhelsms reduced FcγRIIIa expression.

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F-11

HIGH RATES OF TENOFOVIR DOSE ADJUSTMENTS ARE REQUIRED IN ADEFOVIR EXPERIENCED PATIENTS WITH CHRONIC HEPATITIS B: A 4-YEAR PROSPECTIVE STUDY IN 320 PATIENTS

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Tenofovir (TDF) is a popular anti-HBV strategy for both naïve and experienced patients, yet the 4-year effectiveness and safety in field practice patients long-term exposed to Adefovir (ADV) is poorly known. Methods: In a single center, 320 NUC-experienced CHB patients received TDF for 48 months (range 0–85) as a switch from ADV + LAM or LAM or ETV. Virological response was undetectable HBV DNA; safety analysis focused on glomerular (eGFR) and tubular renal function. Results: At baseline age was 59 (24–82), 85% HBeAg-negative, 62% cirrhotic, 88% normal ALT, 74% with undetectable HBV-DNA and 26% with 3.0 log IU/ml (1.1 to >9-0) HBV-DNA. 86% of the patients were switched from ADV + LAM. TDF was started at 300/48 h or lower dose in 29% of the cases. Virological responses progressively increased to 100% at year 4 with normal ALT for most patients. 4% cleared HBsAg and 9 successfully withdrew from treatment. Creatinine and phosphate levels remained unchanged over 4 years of TDF treatment. The same was also true for patients with creatinine >1.5 mg/dl, phosphate <2.3 or <2.0 mg/dl. Because of eGFR decline, TDF dose was reduced to 300/48 h in 57 patients (23%) and 300/72 h in 15, without any HBV-DNA rebound. 19 additional patients (6%) were successfully switched to ETV because of drug-related side effects. Overall, 91 patients (28%) either required a dose reduction or withdrew from TDF for side effects but none developed acute renal failure or Fanconi syndrome or required hemodialysis. The yearly HCC rate was 1.2%, lower than expected based on natural history studies. No patient decompensated. Overall, 7 patients (2%) were transplanted (all for HCC) and 14 (4%) died (7 because of HCC). In conclusion, careful monitoring of glomerular and tubular function and proactive downdosing of TDF minimized the risk of renal toxicity in ADV exposed patient treated for 4 years with TDF monotherapy.

http://dx.doi.org/10.1016/j.dld.2014.01.109
PORTAL HYPERTENSION BUT NOT HCC RISK IS ATTENUATED IN COMPENSATED CIRRHOTICS FOLLOWING 10 YEARS OF TREATMENT WITH NUCLEOS(T)IDE ANALOGS

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**Background and aim:** Whether long-term HBV suppression by nucleos(t)ide analogs (NUC) modifies the course of esophageal varices (EV), a complication that predicts both clinical decompensation and anticipated liver related death in patients with HBV cirrhosis, is currently unknown.

**Patients and methods:** 107 HBeAg-negative cirrhotic patients (56 years, 83% males, 87% Child-Pugh A) with either no EV (n = 80) or F1 (n = 27) treated with LMV ± ADV or TDF for 10 years were studied. Laboratory tests including serum HBV DNA were performed every 3 months, abdominal ultrasound scan every 6 months and upper GI endoscopy according to Baveno guidelines. None of the patients were on beta-blockers prophylaxis.

**Results:** During 109 (12–174) months of treatment, pre-existing EV regressed in 17 (63%) patients, remained unchanged in 8 (30%) and increased in size in 2 (7%), whereas de-novo EV developed in 5 (6%) (4 F1 and 1 F2) of the 80 EV-free patients at baseline. Overall, the 10-year rates of EV regression, progression and de novo onset were 80%, 12% and 8%. Six of 7 (86%) EV progressors had either a clinical breakthrough due to LMV-R and/or developed a hepatocellular carcinoma (HCC). While no patient bled from ruptured EV, 28% (2.8% per year). The 10-year cumulative rates of overall and liver-related survival were 89% and 91%, respectively.

**Conclusions:** Long-term suppression of HBV by nucleos(t)ide analogs minimizes the risk of de-novo EV and EV progression in compensated cirrhotics, leaving however HCC incidence rates unaffected.

http://dx.doi.org/10.1016/j.dld.2014.01.110
Introduction and aim: Entecavir antiviral potency, efficacy in NUC experienced patients and liver complications in cirrhotic patients was assessed in daily clinical practice.

Materials and methods: 423 patients, enrolled from 2009 to 2011 (median 19, range 3–54 months follow-up) were evaluated for rate of undetectable HBV-DNA, HBsAg seroconversion, drug resistance mutations and monitored for ascites, variceal bleeding and HCC.

Results: 191 (45%) had liver cirrhosis, mean age 57.1 ± 11.05; HCC was present in 41 and 25 developed HCC (median 15, 3–44 months, cumulative rate 5.9% at 3.6 years), ascites resolved in 11/37 cases, while 22 patients developed ascites; 5 variceal bleedings were observed, 18 patients died for liver causes. Rate of undetectable HBV-DNA was 58.2%, 80.2%, 86.6% and 88.8% at 6, 12, 18 and 24 months. Eighteen/57 HBsAg positive patients seroconverted to anti-HBe.

At enrolment 23 patients were on treatment with: LAM (7 patients), LAM + ADV (4), ADV (7), LdT (2), TDF (1) and LAM + TDF (2), while 7 had been previously treated with LAM (3), LAM/ADV (1), ADV (2) and TDF (1). Twenty-three/30 NUC experienced patients started therapy with ETV, 7 with ETV + TDF or ETV + ADV; 23/30 cleared viremia while HBV-DNA ranged between 20 and 154 UI/ml in the remaining 7.

One patient switched to TDF because of virologic failure (L180M, M204V, M250V mutations associated with entecavir resistance), a second subject added TDF because of partial virologic response.

Conclusions: The study of Entecavir treatment in field practice confirms its efficacy in suppressing HBV replication also in NUC experienced subjects. Interim analysis of HCC rate confirms results of other real-life studies.

http://dx.doi.org/10.1016/j.dld.2014.01.111

F-14

PROPHYLAXIS STRATEGY TO PREVENT HBV-REACTIVATION IN ONCOLOGIC IMMUNOSUPPRESSED PATIENTS: RESULTS OF GUIDELINES-BASED ON-FIELD PRACTICE

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Background and aims: The management of patients at risk for HBV reactivation under chemotherapy and/or immunosuppressive therapy is based on current specific guidelines (CG) deriving. Aim of this prospective observational study was to verify the application and effectiveness of national guidelines in our hospital setting.

Methods: From September 2010 to March 2013, all patients presenting at the Oncology Department of our Institution with a new diagnosis of solid tumor were consecutively enrolled. A total of 2,007 pts underwent a serological screening for HBV together with a clinical hepatic staging. According to CG, therapy with third generations NUCs was started for active carriers (HBVDNA > 2000 UI/ml), while universal prophylaxis (UP) with lamivudine was started for inactive carriers (HBVDNA < 2000 UI/ml). HBVDNA, ALT levels and renal function were monitored every 3 months during chemotheraphy and for additional 12–18 months from the last chemotherapy. A 3-months HBsAg monitoring was instituted in HbcAb+ patients.

Results: 86 patients (56 male, 30 female, mean age 66 ± 19 years) resulted HBsAg+ (prevalence 4.5%). 17 were defined active carriers and started on NUCs (4 TDF, 13 ETV). 30 were inactive carriers and started up with lamivudine. 39 patients at the time of initial evaluation were excluded from chemotherapy due to advanced oncological disease. 587 patients were HbcAb+ (prevalence 29.2%). No virological/clinical HBV-reactivation was observed in HBsAg+ patients in 30 months follow-up. No reactivations occurred in HbcAb+ patients.

Conclusion: This prospective observational on-field study confirms:

- The high prevalence of HBV “exposure” in oncological population.
- That the proper application of CG is effective in preventing HBV-reactivation in HBsAg+ and/or HbcAb+ patients who undergo chemotherapy.

http://dx.doi.org/10.1016/j.dld.2014.01.112

F-15

REACTIVATION OF HEPATITIS B VIRUS IN HBsAG-NEGATIVE HBCAB-POSITIVE PATIENTS WITH PSORIASIS UNDERGOING IMMUNOSUPPRESSIVE THERAPY

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Introduction and aim: Viral hepatitis reactivation has been widely reported in patients undergoing immunosuppressive therapy; however, few data are available on the risk of HBV reactivation in patients with psoriasis receiving immunosuppressive drugs.

We conducted a retrospective study to value the effect of immunosuppressive therapy on HBV infection in psoriatic patients.

Methods: The study included all consecutive psoriatic patients who attended an Italian tertiary referral hospital from 2008 to 2012. A total of 412 patients were consecutively enrolled. We evaluated: HBV markers, type of immunosuppressive treatment and the occurrence of HBV reactivation ( reappearance of HBsAg, increase of HBV-DNA at least 1 log in association with or without increase of aminotransferase levels). The observational period ranged from the beginning of immunosuppressive treatment to 12 months after the end of therapy.

Results: A total of 225/412 (54.6%) patients with psoriasis and receiving immunosuppressive therapy (traditional therapy with cyclosporine or methotrexate, and/or biological drugs, such as adalimumab, infliximab, etanercept, golimumab, ustekinumab) were tested for HBV infection. We identified 23/225 subjects (10.2%) with isolated HbcAb positivity and 36/225 (16%) with HBsAb/HbcAb positivity. No patient was HBsAg positive. No patient underwent preemptive therapy with Lamivudine or other antiviral drugs. No patient showed episodes of HBV reactivation.

Conclusions: The immunosuppressive therapy in patients HbcAb positive with or without HBsAb positivity, in dermatological setting seems to be safe, regardless to the type of treatment (biological and/or traditional).

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HEPATITIS B VIRUS SCREENING IN HEALTHY IMMIGRANTS: A STRATEGY TO PREVENT THE SPREAD OF INFECTION AND TO IDENTIFY AND MANAGE CHRONIC HEPATITIS B (CHB)

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Introduction: Screening for hepatitis viruses is a form of secondary prevention aimed at early disease detection so antiviral treatment can be begun precociously and liver disease can be prevented. Globalization is radically changing epidemiology patterns and the way transmissible viral diseases are spread.

Aim: The aims of this study were to assess the prevalence of HBV in immigrants residing in Padua (Italy).

Materials and methods: Regular healthy immigrants were sent to our clinic by community leaders from March 2013 to October 2013, questioned about their sociodemographic characteristics, tested for HBcAb and, if positive, for HBsAg. HBsAg +ve subjects were studied for HBVDNA levels and enrolled for clinic controls of liver disease.

Results: 450 (264 – M 58.7% and 185 – F 41.3%) immigrants were screened. 35% were from Eastern Europe, 23% from Asia, 36% from Africa, and 2% from other areas. This distribution is comparable with immigrants residing in Padua. 144 (32%) were anti-HBcAg +ve, 41 (1%) HBsAg +ve subjects were studied for HBVDNA levels and enrolled for clinic controls of liver disease.

Conclusions: Hepatitis B virus screening on healthy immigrants in our area is effective to identify HBsAg +ve subjects and seems to be able to define the number of the patients with HBV related liver disease. Management and treatment of CHB in the identified subjects can prevent cirrhosis and HCC, changing the natural history of HBV chronic infection.

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KEY GENETIC MARKERS IN THE FULL-LENGTH HBSAG GENE CORRELATE WITH HBV-DRIVEN CARCINOGENESIS BY AFFECTING HBSAG SECRETION AND RELEASE

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**Background:** Intracellular HBsAg-retention can favor the onset of hepatocellular-carcinoma (HCC). Here, we define HBsAg mutations correlated with HBV-induced HCC in-vivo, and investigate their impact on HBsAg-retention and secretion in an in-vitro model.

**Methods:** Sixty-nine HBV chronically infected patients (74.6% genotype-D; 20.9% genotype-A; and 4.5% others), 21 with HCC and 48 asymptomatic (as control), are analyzed. Associated HBsAg mutations with HCC are assessed by Chi-Squared test with Benjamini–Hochberg for multiple-comparison correction of HBsAg-mutations with HCC is assessed by Chi-Squared test with Benjamini–Hochberg for multiple-comparison correction.

**Results:** Novel HBsAg-mutations (N40I-K141N-P203Q-S210R) correlate with HCC development (P = 10−2 to 10−3). 19/21 patients with HCC carry ≥ 1 of them (range-prevalence: 12.5–37.5%), while these mutations are absent (0/48 for N40I) or occurring with low frequency (1.9% for K141N-P203Q, 7.5% for S210R) in non-HCC patients. Strong correlations are observed for P203Q + S210R (phi = 0.83) and N40I + K141N (phi = 0.56).

In vitro experiments, the pair P203Q + S210R, localized in the membrane-embedded C-terminal HBsAg-domain critical for HBsAg-secretion, drastically decreases HBsAg-release in supernatant compared to P203Q and S210R alone, and to wild-type (9.42[sd ± 1.35] IU/ml for P203Q vs. 26.85[sd ± 13.01] IU/ml for P203Q + S210R; 34.40[sd ± 13.28] IU/ml for WT, P < 0.01). P203Q + S210R association also decreases the ratio of supernatant to lysate HBsAg compared to wild-type (0.61 for P203Q + S210R versus 1.20 for wild-type), further supporting their ability in inducing HBsAg intracellular-retention.

Similarly, K141N + N40I strongly decreases supernatant HBsAg-levels compared to wild-type and K141N alone (4.67[sd ± 2.23] IU/ml for K141N + N40I; 13.43[sd ± 9.68] IU/ml for K141N; 28.25[sd ± 13.12] IU/ml for WT, P < 0.05). N40I resides in HBsAg cytosolic-loop known to be important in HBsAg-secretion.

**Conclusions:** Key HBsAg genetic-elements correlate with HCC by inducing intracellular HBsAg-retention. Their detection may help identifying patients at higher HCC-risk that may deserve more intensive liver evaluation, and/or earlier anti-HBV therapy.

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**F-19**

ALTERATION IN LIPID METABOLISM AFTER AN ORAL FAT LOAD IN NON-ALCOHOLIC FATTY LIVER DISEASE

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**Introduction and aims:** Non-alcoholic fatty liver disease (NAFLD) is associated with insulin resistance (IR) and impairment in whole body lipid metabolism leading to hepatic fat accumulation and fibrosis. We investigated the effect of a lipid load on lipolysis and hepatic glucose metabolism in non-diabetic/non-NAFLD patients.

**Materials and methods:** [2H5]glycerol and [2H2]glucose kinetics, plasma levels of glucose/insulin, lipid profile, endogenous glucose production (EGP) and lipolysis (Glycerol Ra) were determined in 20 patients with biopsy proven NAFLD and 9 controls during an oral fat load (200 ml dairy cream and an egg yolk). Peripheral/hepatic/adipose tissue-IR indices were derived from plasma glucose/insulin, EGP and lipolysis. Subcutaneous, visceral and hepatic fat were assessed by NMR.

**Results:** During fasting, EGP and lipolysis were similar in NAFLD patients and in CT (8.9 ± 1.2 vs. 8.7 ± 0.9 μmol/kg min and 2.4 ± 0.9 vs. 2.1 ± 0.6 μmol/kg min) although indices of IR were higher in NAFLD patients (HOMA 2.3 ± 0.9 vs. 1.3 ± 0.4, Hep-IR 92 ± 34 vs. 52 ± 18, Adipo-IR 21 ± 10 vs. 11 ± 5 all p < 0.03). After lipid load, the triglycerides (TG) increase in NAFLD was 2-folds than CT (iAUC-TG, p < 0.001) while FFA concentration increased similarly. Lipolysis was suppressed in CT but was unchanged in NAFLD, suggesting a remarkable Adipo-IR at low insulin levels that was directly related to NAS score and degree of fibrosis (p < 0.01 for both). Adipo-IR was increased proportionally to fat accumulation in subcutaneous, visceral and hepatic fat (r² = 0.163, p < 0.01; r² = 0.321, p < 0.001; r² = 0.24, p < 0.001, respectively).

**Conclusions:** The metabolic handling of an oral fat load is impaired in subjects with NAFLD independent of pre-existing dia-

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1 Joint first authorship.
2 Joint last authorship.

**CrossMark**
ALTERATION IN GLUCOSE METABOLISM AFTER AN ORAL GLUCOSE LOAD IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Introduction and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with insulin resistance (IR) for different substrates and in different sites. The purpose of this study was to focus on IR for glucose metabolism and its relationship with liver damage.

Materials and methods: We used [3H2]glucose to calculate endogenous glucose production (EGP) in the fasting state and during an oral glucose load (OGTT) enriched with U-13C-glucose to further assess glucose absorption and clearance. Twenty-five non-diabetic/non-dyslipidaemic patients with biopsy proven NAFLD and 10 controls (CT) were studied.

Results: During OGTT, the glucose curves of NAFLD patients were comparable to controls; however, the insulin curves were 1.5-fold higher (AUC-insulin p < 0.05) with a stepwise increase according to the degree of NAS score (p < 0.05) and liver fibrosis (p < 0.01). Intestinal glucose absorption was similar in the two groups. Glucose clearance (GC) was significantly impaired in NAFLD subjects (AUC-glucose clearance decreased 2-folds, p < 0.001). However, when we split GC into muscle and adipose tissue components, only muscle GC was defective and directly related to NAS score and fibrosis (p < 0.05 for both) independent of insulin levels. On the other hand, insulin efficiently suppressed EGP in the liver in both groups.

Conclusions: In non-diabetic/non-dyslipidaemic NAFLD patients post-load glucose clearance is significantly impaired in the muscle and is associated with liver fibrosis, while insulin action in the liver is still preserved. Muscle IR is a primary defect in NAFLD and contributes to liver damage.

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OBSTRUCTIVE SLEEP APNEA SYNDROME IN NON-SEVERELY OBESE PATIENTS WITH NAFLD

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Introduction: We previously showed that a third of non-severely obese patients with nonalcoholic fatty liver disease (NAFLD) are at risk of obstructive sleep apnea syndrome (OSAS), that in the presence of daytime somnolence was independently associated with non-alcoholic steatohepatitis (NASH) and fibrosis severity.

Aim: Aim was to evaluate the prevalence of OSAS by polysomnography in non-severely obese NAFLD patients, and the correlation with hepatic damage and cardiovascular risk factors.

Materials and methods: We enrolled 22 consecutive patients (M/F 15/7, age 51 ± 12 years, BMI 29.7 ± 3.8 kg/m²) with histological diagnosis of NAFLD, absence of other liver diseases, without a previous diagnosis of OSAS. As controls, we evaluated healthy subjects (n = 10) with comparable age, sex and BMI distribution. Subjects underwent polysomnographic study, which was analyzed by a single physician experienced in sleep disease.

Results: Seventeen/22 patients (77%) vs. 1/10 matched controls (10%) were positive for OSAS (p = 0.003). Moderate-to-severe OSAS (apnea hypopnea index AHI > 15) was observed in 7 cases (31%) and mild OSAS (AHI 5–15) in 10 (45%). Presence of moderate-severe OSAS (indication for treatment) was associated with hypertension (p = 0.005, p = 0.001 after correction for BMI) and diabetes or impaired fasting glucose (p = 0.007, p = 0.004 after correction for BMI). After correction for BMI and ALT levels, there was a trend for correlation between moderate-severe OSAS and fibrosis > 1 (p = 0.09).

Conclusions: Preliminary data suggest that non-severely obese patients with NAFLD have a higher prevalence of OSAS than healthy subjects with comparable demographic features and body mass. Moderate-severe OSAS may increase cardiovascular risk by driving adrenergic activation.

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MARGINAL LIVER GRAFTS CHARACTERIZATION:
(1) IMAGING AND IN SITU SPECTRAL ANALYSIS OF NILE RED-INDUCED LIPID FLUORESCENCE IN MCD-INDUCED LIVER STEATOSIS

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Reclaiming fatty liver grafts for transplantation appears mandatory due to the shortage of donors. We demonstrated that obese Zucker rat livers were damaged by conventional cold-storage, but were better preserved by subnormothermic machine perfusion (Vairetti et al., 2009). Steatosis induced by a Methionine-Choline deficient (MCD) diet in the liver of Wistar rats is being now investigated respect to an isocaloric diet. We characterized the lipid content using the fluorescent probe Nile Red (NR): under excitation in the 400–500 nm range, NR bound to triglycerides or cholesteryl esters emits a bright yellow fluorescence and a dimmer red emission when bound to phospholipids. 1–4 and 9 weeks of MCD or control diet were considered. Unfixed cryostat liver sections were stained with a 1.6 µM solution of NR in glycerol. Areas occupied by yellow-emitting neutral lipid droplets were quantified by image analysis using a fluorescence Zeiss Axioscope 2 Plus microscope, a Canon EOS 1100D digital camera and Image Pro Plus 4.5 software. Spectral analysis was performed by microspectrofluorometry, using a Leitz microspectrograph with an EG&G Optical Multichannel Analyzer (excitation: 436 nm). Spectra were normalized for a direct comparison of the emission profile, and hence of the lipid nature (triglycerides: 530–620 nm; phospholipids: 630–700 nm). Control livers contained yellow-fluorescent micro lipid droplets whose area increased steadily from the 1st to the 4th week and stabilized henceforth. Response to MCD was highly variable in terms of yellow-fluorescent areas. Severe macrosteatotic changes occurred from the 2w treatment onwards as demonstrated by the several-fold increase in yellow emission-positive areas. In MCD-treated animals, spectral analyses revealed a strong relative increase of the yellow but also of the red signals respect to the controls. The latter suggest abundance of phospholipid-bound vesicles, consistent with autophagocytosis that will be investigated at the EM level.

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IN VITRO INDUCTION OF VESICULAR STEATOSIS IN AN EPIGENETIC CHANGES ASSOCIATED WITH THE IN VITRO CELLULAR MODEL

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Background and aim: The excessive accumulation of triglyceride-containing lipid droplets (LDs) within hepatocytes in NAFLD patients is a potentially reversible process, although it may evolve into non-alcoholic steatohepatitis (NASH) and eventually cirrhosis and HCC. Here we investigated the epigenetic changes associated with the induction of vesicular steatosis in an in vitro cellular model and the possible role of the Polycomb group Enhancer of Zeste Homolog 2 (EZH2) histone methyl-transferase.

Methods: DMSO-differentiated human non-transformed hepatocytic HepaRG cells treated with oleic acid were used as a cellular model for the induction of vesicular steatosis.

Results: dHepaRG cells treated with oleic acid showed: (a) an increased lipid accumulation and intracellular reactive oxygen species (ROS) generation as compared to normal cells; (b) deregulated lipid metabolism and liver-specific genes, such as PDK4, PLIN4, SLC2A1 and ALB, ALDOB; (c) activated an intracellular inflammatory response, as demonstrated by the upregulation of IL6, IL8, OAS1, NFKB and phosphoSTAT3 levels. We also found that several STAT3/IL6 responsive miRNAs, including miR21 and mir24, are upregulated after lipid overload, paralleling STAT3 activation. Protein and transcripts levels of the Enhancer of Zeste Homolog 2 (EZH2) histone methyl-transferase, that methylates Histon3-K27 and imposes transcriptional repression, were also increased after oleate treatment and ChIP assays showed that the amount of promoter bound H3-K27 trimethylated correlates with the oleate-dependent transcriptional deregulation of the target genes studied.

Conclusions: Epigenetic modifications are involved in steatotic process and they may represent ideal molecular markers for diagnosis and therapeutic target.

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In particular, in NAFLD PBMCs and liver tissues, we appreciated the modulation of Krüppel-like Factor 15 (KLF15) and Tafazzin (TAZ) that were timely correlated with both heart and liver damages progression, evidenced by histologic features and hepatic enzymes (e.g. ALT, AST) serum levels, respectively.

Conclusion: Our data suggest liver as “primum movens” of the cardiovascular damage related to Mets. Furthermore, the correlation between KLF15 and TAZ to heart and liver function, give a new tool to clinicians to assess CVD risk.

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PREGNANCY AND PRIMARY BILIARY CIRRHOSIS: A CASE–CONTROL STUDY

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Although very old papers reported a supposed alterations in sexual hormones, there are no specific reports on pregnancy in PBC.

Aim: To analyze fertility in PBC and to investigate the outcome of pregnancy and the influence of pregnancy on the disease course.

Methods: 233 consecutive female patients with PBC were included in the study (mean age at diagnosis of 52.9 ± 12 years). Among them, 186 had at least one conception and were matched with a 1:2 group of 367 healthy women with at least one conception in their life.

Results: There were 507 pregnancies in PBC patients and 700 in controls (mean average 1.91 vs. 2.73, p < 0.05). The life history in terms of miscarriages, voluntary interruption of pregnancy, term and preterm delivery was similar in the two groups. The number of caesarean deliveries was lower in PBC patients than in controls (8.6% vs. 15.5%, p < 0.05). Perinatal and postnatal death and child birth complications were observed only in the PBC patients (total number of babies = 11 [2.7%]). Pruritus during pregnancy was recorded in 13 patients with PBC (3.0% of total pregnancies) and in none of the control subjects. The risk of pruritus in PBC was associated to the advanced disease (stage III–IV: OR 78, 95% CI 5.55–1108.61, p < 0.05). The risk of miscarriage, corrected for age at pregnancy and number of previous miscarriages, was inversely associated with the histological stage (I–II: OR 0.32, 95% CI 0.10–0.97, p < 0.05). Eight pregnancies occurred after the diagnosis of PBC in 6 patients (two of them with histological stage IV). All pregnancies had a favorable course at term; ursodeoxycholic acid was continued and no worsening of the disease was observed.

Conclusions: Successful completion of pregnancy is a realistic expectation for PBC patients; monitoring of pregnancy and delivery, however, is required due a potential risk of child birth complications.

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TUMORIGENIC POTENTIAL OF CANCER STEM CELLS (CSCS) ISOLATED FROM HUMAN CHOLANGIOCARCINOMA (CCA) SUBTYPES

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Two different forms of CCA have been recently considered. A pure mucin-secreting form (Mucin-CCA) located either intra or extra-hepatically and a Mixed-CCA form with a peripheral location in the liver. We previously identified different subpopulations of CSCs in both Mucin- and Mixed-CCA, including epithelial (CD133+, LGR5+, EpCAM+), mesenchymal (CD90+) and quiescent (CD13+) CSCs. Aim: To evaluate the tumorigenic potential of different sub-population of CSCs isolated from human Mucin- and Mixed-CCA.

Methods: CSC subpopulations were immunoselected from human CCA samples (n = 18 surgically resected patients) and from primary cultures of human CCAs. The tumorigenic potential of CSC subpopulations was tested either in vitro (spheroid formation) or in vivo in xenografted tumors in mice after subcutaneous or intrahepatic injection in normal or cirrhotic (CCL4-induced) livers. In vitro, epithelial CSCs (CD133+, EpCAM+, LGR5+) formed a higher (p < 0.01) number of spheroids with respect to mesenchymal (CD90+) or quiescent (CD13+) CSCs. By comparing Mixed and Mucin-CCA, CD13+ cells immunoselected from IH-mixed and CD90+ or CD13+ cells from Mucin-CCA formed the highest number of spheroids. In subcutaneous tumor xenografts, cancers with a large predominance of stromal markers (desmin, vimentin and α-SMA) were formed by mesenchymal (CD90+), quiescent (CD13+) or epithelial (CD133+) CSCs, while EpCAM+ and LGR5+ only rarely formed tumor xenografts. In intrahepatic xenografts, in contrast, cancers with predominance of epithelial features were formed. Remarkably, injection of CD133+ cells in the cirrhotic liver reproduced the pure Mucin–CCA while CD90+ CSCs formed an undifferentiated CCA. In conclusion, heterogeneous CSC subpopulations were represented in human CCA. They generate different type of cancers depending from the type of CSC and the microenvironment. Remarkably, we identified a single CSC subpopulation capable to reproduce human Mucin–CCA (CD133+) or undifferentiated CCA (CD90+) when injected in the cirrhotic livers.

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QUANTIFICATION OF FIBROSIS IN CHRONIC HEPATITIS C: PERFORMANCE OF NONINVASIVE ELASTOGRAPHY AND ACUSTIC RADIATION FORCE IMPULSE USING COLLAGEN PROPORTIONATE AREA AS REFERENCE

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Background: Liver stiffness, measured by transient elastography (TE) or by acoustic radiation force impulse (ARFI), is proportional to the amount of fibrosis but also affected by necroinflammation. Since staging of fibrosis on biopsy is semiquantitative and reported by classes, collagen proportionate area (CPA), a continuous histological variable measuring collagen unaffected by necroinflammation, could correlate more proportionally with TE and ARFI values.

Methods: Ninety-three consecutive patients with chronic hepatitis (CCHC) were evaluated for histological fibrosis (METAVIR system). In 69 patients, transient elastography (TE) or acoustic radiation force impulse (ARFI) was performed. Liver stiffness was measured with a transient elastography (Fibroscan, Echosense) device (TE) and an acoustic radiation force impulse (ARFI) ultrasound system (iU22, Philips, Bothell, WA, USA) with a formula: Fibroscan-4,5/months between OLT and Fibroscan (assuming HCV infection). TE was unreliable in six patients (6.4%), while ARFI was feasible in all. By linear regression analysis, TE correlated better than ARFI with CPA as a continuous variable (CPA-TE: R² = 0.522, p < 0.001; CPA-ARFI: R² = 0.454, p < 0.001) and with CPA quartiles (CPAQ) (CPAq-TE: R² = 0.434, p < 0.001; CPAq-ARFI: R² = 0.334, p = 0.045). By comparison of ROC curves, the performance of TE in predicting CPA ≥ 10.5% (CPAq 3-4) and CPA > 15.4% (CPAq 4) was significantly higher than that of ARFI (AUROC: 0.884 vs. 0.764; p = 0.006 and AUROC: 0.863 vs. 0.757; p < 0.001, respectively). At univariate analysis, age, ALT, AST, GGT, platelets, TE, ARFI and inflammation grade were related to CPAq 3-4. At multivariate logistic regression analysis, only TE (OR: 1.67, 95%CI: 1.13-2.48, p = 0.010) was independently related to CPAq 3-4. Similarly, AST, ALT, GGT, platelets, TE, ARFI and inflammation grade were related to CPAq 4, but by multivariate analysis only TE (OR 1.39, 95%CI: 1.01-1.76, p = 0.040) was independently associated with CPAq 4 (15.4%). The Metavir grade of inflammation was marginally associated with CPA ≥ 15.4% (OR: 4.14, 95%CI: 0.98-17.56, p = 0.064).

Conclusions: TE measurements seem to provide prognostic information on mortality after OLT.

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MORTALITY PREDICTION WITH TRANSIENT ELASTOGRAPHY AFTER LIVER TRANSPLANTATION FOR LIVER FAILURE OF VARIOUS AETIOLOGIES

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Background: Transient elastography (TE, Fibroscan, Echosense) has been widely validated in the setting of transplant patients with recurrent hepatitis C (HCV). Few data exist about the value of TE in detecting liver damage in other aetiologies, and no data exist about the prognostic role of TE after OLT for all aetiologies.

Aim: To evaluate the prognostic value of TE in patients submitted to OLT for liver failure of various aetiologies.

Methods: We retrospectively analyzed 150 liver transplant patients: 94 HCV and HBV-HCV related cirrhosis, 56 non-HCV related cirrhosis (19 HBV, 14 alcoholic, 11 autoimmune disease, 12 mixed causes) who underwent TE measurements between January and August 2007. Patients were divided according two different liver stiffness cut-off as follows: 7.4 kPa which has been reported to be accurate predictor of liver graft damage independently of HCV recurrence and 10.1 kPa which is associated with the best sensitivity and specificity in predicting significant fibrosis in post-transplant HCV recurrence.

Results: During a median follow period of 92 months, 37 patients were still alive and 113 were dead. Both HCV and non-HCV patients with TE values > 10.1 kPa had a lower survival rates than patients with TE values ≤ 10.1 kPa (p = 0.004 and p < 0.0001 respectively). Indeed only HCV patients with a LS > 7.4 kPa had a lower survival rate than patients with TE values ≤ 7.4 kPa (p = 0.008), while no prognostic role was observed in non-HCV patients using this LS cut-off (p = 0.165). Applying for each patients the formula: Fibroscan-4,5/months between OLT and Fibroscan (assuming 4.5 kPa as LS value indicative of no fibrosis) the ROC curve analysis showed a moderate accuracy in predicting survival comparable with Fibroscan AUROC (0.7 range 0.6-0.8, for both).

Conclusion: TE measurements seem to provide prognostic information on mortality after OLT.

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POINT QUANTIFICATION ELASTOGRAPHY AS A NONINVASIVE TECHNIQUE FOR QUANTIFICATION OF LIVER FIBROSIS

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Point-quantification elastography (PQE) is a new shear wave-based elastography technique to assess liver fibrosis (LF) by measuring liver stiffness (LS) noninvasively. LS is expressed in Young’s modulus. Aim of this single-center study was to assess the diagnostic accuracy of PQE in patients with chronic liver disease (CLD) using liver biopsy (LB) as the reference standard. Between September 2012 and May 2013, we enrolled 123 consecutive patients (64 M, 59 F; mean age 50 ± 13) scheduled for LB by referring physicians. On the same day, PQE using the ultrasound (US) system iU22 (Philips, Bothell, WA, USA) and US-assisted LB were performed. 10 PQE measurements were recorded, average-LS (PQE-LS) was calculated. LF was staged according to the METAVIR system. In 69 patients, transient elastography (TE) data were also available. Aetiologies of CLD were: HCV (57) or HBV-infection (21), alcohol (2), non-alcoholic-steatohepatitis (10), autoimmune hepatitis (3), primary biliary cirrhosis (2), primary biliary cholangitis (1), undefined (14) or a combination of the above aetiologies (13). PQE-LS was significantly correlated with LF (r = 0.647, p < 0.001). Optimal cut-off values, sensitivity (se) and specificity (sp) for the different levels of LF were determined by
analysis of receiver operating characteristic (ROC) curve: 4.7 kPa for mild LF (F1) (se 63.7%, sp 77.8%), 6.5 kPa for moderate LF (F2) (se 75.0%, sp 86.4%), 7.3 kPa for severe LF (F3) (se 88.6%, sp 86.2%) and 10.2 kPa for cirrhosis (se 89.5%, sp 83.5%). There was a statistically significant correlation also between PQE-LS and TE-LS (r = 0.796, p < 0.001). In patients with PQE and TE data, the diagnostic performance of the two techniques was assessed by the area under the ROC curve (AUC) analysis for F0 versus F1–F4, F0–F1 versus F2–F4, F0–F2 versus F3–F4 and F0–F3 versus F4. AUCs were: 0.70 (95% confidence interval[CI]: 0.51–0.89) for PQE and 0.73 (95%CI: 0.61–0.86) for TE, 0.89 (95%CI: 0.81–0.97) for PQE and 0.91 (95%CI: 0.85–0.98) for TE, 0.91 (95%CI: 0.83–1.00) for PQE and 0.91 (95%CI: 0.84–0.99) for TE and 0.88 (95%CI: 0.80–0.96) for PQE and 0.85 (95%CI: 0.73–0.97) for TE when comparing F0 versus F1–F4, F0–F1 versus F2–F4, F0–F2 versus F3–F4 and F0–F3 versus F4, respectively. Differences among PQE and TE AUCs were not statistically significant.

Conclusions: PQE is a reliable noninvasive method to assess LF, with a diagnostic performance not significantly different from TE and with the advantage of compute measurements visualizing in real-time the explored area.

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THE AMPK-RELATED KINASE, NUAK2 IS MODULATED BY THE ACTIVATION PROCESS AND REGULATES MOTILITY OF HEPATIC STELLATE CELLS (HSC)

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Background: AMPK related kinases (ARKs) are energy sensors and controllers of cellular structure. Nuak2 is a novel member of this family, and has different effects on cell motility and cytoskeletal organization depending on the cell types. AMPK is expressed in HSC, and negatively modulates their profibrogenic features. However no information is available on the involvement of Nuak2 during HSC activation or in the biology of these cells.

Aim of this study was to investigate whether Nuak2 is expressed in HSC and its possible involvement in HSC activation and functions.

Methods: HSC were isolated from normal rat liver and activated by culture on plastic in the presence or absence of AICAR, an AMPK activator. Knockdown of Nuak2 was achieved by siRNA. Cell migration was evaluated in modified Boyden Chambers.

Results: Incubation with AICAR, an activator of ARKs, blocked the activation process of primary HSC and caused disruption of the actin cytoskeleton, resulting in altered cell adhesion and spreading, but without effects on viability. HSC were found to express the alpha1 and the alpha2 subunits of AMPK, but also the Nuak2, a recently characterized ARK. Expression of AMPK subunits and of Nuak2 was high in freshly isolated HSC and decreased during trans-activation to a myofibroelastic phenotype. In fully activated HSC, down-regulation of Nuak2 was associated with a marked increase in cell migration, together with changes in the expression of several molecules implicated in cytoskeletal organization.

Conclusions: ARKs, including Nuak2, are modulated during the activation process of HSC and regulate cytoskeletal organization and cell motility.

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EFFECTS OF NON-SELECTIVE Β-BLOCKERS ON HEMODYNAMIC AND PARACENTESIS INDUCED CIRCULATORY DYSFUNCTION IN CIRRHOTICS UNDERGOING LARGE VOLUME PARACENTESIS

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Introduction: Non-Selective Β-Blockers (NSBB) have been associated with increased incidence of Paracentesis Induced Circulatory Dysfunction (PICD) and reduced survival in patients with cirrhosis and refractory ascites.

Aim: To prospectively evaluate effects of NSBB on intra-individual central and peripheral hemodynamic changes after large volume paracentesis (LVP) and incidence of PICD, defined as increase of Plasma Renin Activity (PRA)> 50% post-paracentesis.

Methods: Patients with cirrhosis and refractory ascites, having indication to initiate or discontinue NSBB, were enrolled. During 2 consecutive LVP (while been respectively on and off NSBB therapy), each patient underwent measurement of cardiac output (CO), systemic vascular resistances (SVR), peripheral vascular resistances (PVR) using impedance cardiography and plethysmography. PRA was obtained before and 60 min after LVP for every procedure.

Results: Eleven patients with diuretic-resistant refractory ascites were enrolled, 6 completed the study; in all patients there was introduction of propranolol (40 mg twice/daily). Before NSBB initiation, SVR (1808 ± 358.3 vs. 1398 ± 332.4 dyn cm⁻²; p = .02) and PVR (45.9 ± 7.0 vs. 27.7 ± 5.9 mmHg min dl ml⁻¹; p = .04) significantly decreased 60 min after LVP than pre-paracentesis; CO consequently showed increasing trend (3.8 ± 0.67 vs. 4.4 ± 1.141/min; p = .06). PICD was diagnosed in 2/6 patients. While on NSBB therapy, CO did not increase after LVP (3.3 ± 1.0 vs. 3.6 ± 1.01/min; p = .1), but this was counterbalanced by a smaller decrease of SVR (1981.12 ± 314.2 vs. 1763.29 ± 555.05 dyn cm⁻²; p = .1) and PVR (44.17 ± 12.2 vs. 32.1 ± 7.86 mmHg min dl ml⁻¹; p = .2). Three of six patients developed PICD.

Conclusions: The inotropic negative effect of NSBB could be counterbalanced by smaller decrease of vascular resistances probably due to splanchnic and peripheral β-blockade. Incidence of PICD seemed not to be increased by NSBB which may be void of detrimental effects in cirrhotics undergoing LVP.

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Spleen stiffness (ss) can represent an indirect and safe method to detect portal hypertension and severity of oesophageal varices (OV).

Aim: Investigate the role of SS, assessed by ARFI (acoustic radiation force impulse) to predict the grade of severity of OV.

Methods: We enrolled 48 cirrhotic patients (age 61 ± 12; mean ± SD) (group A), 18 patients with chronic liver disease (age 46 ± 15; mean ± SD) (group B) and 14 healthy subjects (age 40 ± 4; mean ± SD) (group C). Endoscopy was performed only in cirrhotic patients. Right liver stiffness (rls), left liver stiffness (lls) and SS were assessed ARFI by the same expert operator.

Results: ARFI (mean value m/s ± SD): Group (A); 3.16 ± 0.41 (rls), 3.02 ± 0.47 (lls), 3.48 ± 0.46 (ss). Group (B); 1.14 ± 0.11 (rls), 1.22 ± 0.22 (lls) and 2.29 ± 0.24 (ss). Group (C); 1.19 ± 0.15 (rls), 1.84 ± 0.41 (lls) and 2.67 ± 0.44 (ss) (p < 0.0001) between the groups. A linear correlation was found between (ss) and (rls) in all the groups r = 0.805, p < 0.001. We divided cirrhotic patients in 4 groups: patients without varices, patients with varices F1, patients with varices F2, patients with variceal red signs (RS). For each group we obtained the mean value ± SD of (ss): no varices 2.82 ± 0.6, F1 3.51 ± 0.26, F2 3.47 ± 0.38, RS 3.73 ± 0.36. We found significant differences between the group (p < 0.001). We produced a ROC curve comparing varices with and without (RS) (ss p < 0.01), AUROC: 0.72, p = 0.01. The best cut off of (ss) to predict RS presence was 3.5 m/s, with sensitivity of 90% and specificity of 53%.

Conclusions: Our results demonstrate a strong correlation between spleen stiffness, stage of oesophageal varices and probability of bleeding.

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PREDICTION OF EVS PRESENCE BY CALCULATED RISK SCORES IN B—VIRAL CIRRHOSIS

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Introduction: Non-invasive predictive models have been proposed to predict clinical significant portal hypertension in cirrhosis.

Aim: To compare the diagnostic accuracy of non invasive combined tests previously published for identifying esophageal varices (EV) in our cohort of HBV compensated cirrhosis.

Materials and methods: Sixty-one patients (mean age 59.6 ± 9.76) treated or ongoing antiviral therapy (42/61) were analyzed. All patients had spleen diameter, liver stiffness and endoscopy. Portal pressure (HVPG) was performed in 13. We evaluated the accuracy of liver stiffness (LS), platelet count to spleen size (PSD), LS x spleen size/platelet count (LSPS) and varices risk score (VRS) in predicting EV. Endoscopy was used as goal standard.

Results: LS was not accurate enough to predict the presence of varices. LSPS and VRSs accurately estimated the presence of varices (area under ROC curve, AUROC, 0.935 and 0.922, CI 0.841–0.982 and 0.824–0.975 respectively, p < 0.0001) and were comparable (p = 0.583). Their performance was higher than PSD (AUROC 0.901, CI 0.797–0.963, p < 0.0001). LSPS score < 1.5 had negative predictive value of 100% to identify patients without EV and accurately classified patients with HVPG < 5 mmHg.

Conclusions: LSPS is a good predictor of EV in compensated HBV cirrhosis and might reduce the need of endoscopy. A LSPS cutoff < 1.5 appeared useful to identify patients with very early stage of liver disease. Its role in predicting risk of liver-related complications during long term antiviral therapy should be investigated.

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EVIDENCE OF AN IPERACTIVATION OF INFLAMMASOME IN PERIPHERAL BLOOD MONONUCLEATED CELLS OF PATIENTS WITH ACUTE ON CHRONIC LIVER FAILURE

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Background and aims: Acute on chronic liver failure (ACLF) has been recently defined by the failure of one or more organs and by a high short term mortality in patients who are hospitalized for an acute decompensation of cirrhosis. It has been suggested that inflammation may be relevant in the pathophysiology of ACLF. We aimed to evaluate a possible role of inflammasome iper-activation in the pathogenesis of ACLF.

Methods: Seventy-two consecutive patients who were hospitalized for an acute decompensation of cirrhosis were included. In 21 of them (29.2%) an ACLF was diagnosed. Gene expression levels of NF-κB, caspase-3, caspase-1, TNF-α and IL-1β were detected in peripheral blood mononucleated cells (PBMCs) from ACLF and no-ACLF patients. In order to compare data between patients with and without ACLF no-parametrical analysis (Mann-Whitney test) was performed.

Results: The plasma levels of TNF-a and IL-6 were found to be significantly higher in patients with ACLF than in those without ACLF (p < 0.05, p < 0.05, respectively). Gene expression levels of NF-κB (p = 0.03), caspase-3 (p = 0.03), caspase-1 (p = 0.05), TNF-α (p = 0.05) and IL-1β (p = 0.05) resulted significantly higher in PBMCs from ACLF vs. no-ACLF patients. The rates of bacterial infection as precipitating event of ACLF did not differ statistically in a significant way between the 2 groups of patients (52.4% vs. 37.3%, respectively, p = NS).

Conclusions: Our data show that the effector pathway (NF-κB, caspase-1 and IL-1β) is significantly over-expressed in PBMCs from patients with ACLF vs. no-ACLF. This may contribute to the pathophysiology of inflammation in these patients.

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SCCA-IGM IS PREDICTIVE OF HEPATOCELLULAR CARCINOMA DEVELOPMENT IN PATIENTS WITH HCV CIRRHOSIS—A PROSPECTIVE STUDY

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Introduction and aims: In chronic hepatitis C increasing SCCA-IgM levels were found predictive of fibrosis progression and in HCV cirrhosis high levels of this biomarker were associated with an increased risk of HCC development in a recent multicenter retrospective study. Aim of the study was to assess the clinical significance of SCCA-IgM in cirrhotic patients in a prospective study.

Materials and methods: 71 patients with cirrhosis (M/F: 53/18) were consecutively enrolled and followed up for a median period of 53 months at our Institution. Etiology was HCV in 37%, HBV in 17%, alcohol in 44% and metabolic or unknown in 2% of the cases. The majority of the patients (69%) were Child A. SCCA-IgM was measured in serum at presentation by ELISA (Hepa-IC, Exptagen).

Results: SCCA-IgM was more frequently detected in HCV cirrhosis than in the remaining patients (38% vs. 13%, p = 0.02). During follow up 11/26 HCV patients developed HCC (median time: 11 months). In this group the positivity of the biomarker at presentation was significantly associated with HCC development (70% vs. 25%, p = 0.04), but not with other cirrhosis complications. Kaplan–Meier curves confirmed a lower HCC-free survival in HCV cirrhotic patients positive for SCCA-IgM, compared to negative cases (p = 0.03). The relative risk of HCC development was 2.7 in HCV cirrhosis (95% CI = 1.6–4.6) and increased up to 3.2 (95% CI = 1.1–9.6) in the subset of SCCA-IgM positive patients.

Conclusions: In patients with HCV cirrhosis SCCA-IgM was highly predictive of HCC development and may be considered as a prognostic tool for the subclassification of cirrhotic patients.

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AKIN VS CONVENTIONAL CRITERIA TO DIAGNOSE PRE-LIVER TRANSPLANTATION ACUTE KIDNEY INJURY: GENDER DIFFERENCES AND OUTCOME POST-LT

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Introduction: Acute kidney injury (AKI) has a negative impact on pre and post-liver transplantation (LT) and is associated with increased morbidity and mortality.

Conventional criteria for AKI diagnosis in patients with end-stage liver disease awaiting liver transplantation (ESLD-wLT) include an increase in serum creatinine(sCr) > 1.5 mg/dL (International Ascites Club Criteria). Several studies have confirmed that sCr in patients with ESLD-wLT is influenced by several underlying factors, such as decreased hepatic creatine synthesis and reduced muscle mass. This variability may be magnified in women because for a given level of creatinine, women have a lower glomerular filtration rate than men, evaluated by renal scintigraphy.

AKIN criteria according to Kidney Disease Improving Global Outcomes (KDIGO 2012) have been validated to define and stratify AKI on the basis of deviation of sCr from baseline rather than absolute values.

Aim: The aim of the study was to compare the prevalence of AKI pre-LT in patients with ESLD-wLT based on AKIN vs conventional criteria and the incidence of AKI post-LT.
Materials and methods: This is a single-centre retrospective study of 91 patients (73M/18F), mean age 55 ± 9 years, assessed from listing to first week post-LT (2008–2013). Acute kidney injury was staged applying conventional criteria (sCr > 1.5 mg/dL) and AKIN criteria: increase in sCr ≥ 150% from baseline or ≥0.3 mg/dL in 48 h (Stage 1), sCr ≥ 200% from baseline (Stage 2), sCr ≥ 300% from baseline or sCr ≥ 4 mg/dL (Stage 3).

Results and conclusions: Fourteen patients showed undiagnosed AKI pre-LT according to AKIN vs conventional criteria: ten patients presented stage 1 and four patients stages 2–3. All patients with advanced AKI pre-LT (stages 2–3) were women. Post-transplant AKI occurred in all of them (4/4 patients).

AKIN criteria improve AKI diagnosis as compared to conventional criteria in particular in women: about 50% of patients would have not been recognized outside AKIN criteria. Moreover, AKI diagnosis according to AKIN criteria correlates with occurrence of AKI post-LT.

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3D-SPECKLE TRACKING ECHOCARDIOGRAPHY IN CIRRHOTIC PATIENTS: A POSSIBLE TOOL FOR THE DIAGNOSIS OF CIRRHOTIC CARDIOMYOPATHY

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Introduction: Cirrhotic cardiomyopathy (CCM) is an entity which implies systolic and diastolic dysfunction, electrophysiological abnormalities and myocardial structural changes in patients with liver cirrhosis. Even though the main clinical features of cirrhotic cardiomyopathy have been defined, to date a sensitive diagnostic test that can identify patients with this condition is still lacking.

Aims: The aim of the study was to assess the mechanic of the left ventricle using the 3D Speckle Tracking Echocardiography (3D-STE) in order to find echocardiographic patterns, suitable for the diagnosis of CCM.

Methods: 10 cirrhotics (7 Child-Pugh A and 3 B; mean Meld-Score 11 ± 3; 5 with esophageal varices and 2 with ascites) and 32 healthy subjects were studied using a conventional 2D echocardiography and the 3D-STE technology (Artida 3D wall motion tracking, Toshiba). The 3D-STE is not influenced by the hemodynamic changes occurring in cirrhotic patients. The results were analyzed according with the Guidelines of the American Society of Echocardiography.

Results: Left ventricular mass index and ejection fraction were significantly increased in cirrhotics vs. controls (105 ± 21 vs. 70 ± 14 g/m², p = 0.0003, 62 ± 7 vs. 57 ± 5%, p = 0.009) probably due to the myocardial structural changes and hyperdynamic circulation. According to the 3D-STE, the global peak circumferential left ventricular strain was significantly reduced (24.6 ± 4.5 vs. 27.3 ± 3.4%, p = 0.045), showing a likely impairment of the ventricular mechanical in cirrhotic subjects. The global peak left ventricular twist and the global peak left ventricular torsion tended to be increased, even though these results were not statistically significant (5.2 ± 1.3 vs. 4.1 ± 2.8, p = 0.199; 1.1 ± 0.4 vs. 0.9 ± 1.1, p = 0.559), suggesting an increase of the myocardial’s rotational mechanic in cirrhotics.

Conclusions: 3D-STE allows to identify an intrinsic impairment of the ventricular 3D strain, which may help in the early diagnosis of CCM.

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RENAtal EFFECTS OF DIFFERENT DOSES OF α2-ADRENERGIC AGONISTS, ALONE OR IN COMBINATION WITH DIURETICS, IN RATS WITH EXPERIMENTAL CIRRHOSIS AND ASCITES

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Background: In refractory ascites, adrenergic hyperfunction reduces sodium delivery to the Henle’s loop and causes resistance to diuretics. α2-adrenergic receptor agonists are sympatholytic drugs that may improve natriuresis in advanced cirrhosis.

Aims and methods: Mechanism of action and efficacy of different doses of two α2-agonists (clonidine and SSP-002021R, oral guanfacine prodrug), alone or associated with diuretics, were assessed in eight groups of 10 rats with ascitic cirrhosis due to CCl4 administration (groups G1–G8) and compared to rats with ascitic cirrhosis (G9) and ascitic cirrhotic rats receiving daily diuretics (0.5 mg/kg furosemide + 2 mg/kg K+-canrenoate) (G10). Cirrhotic rats treated with α2-agonists received daily, during the 11–13th weeks of CCl4: clonidine 0.3 mcg (G1), SSP-002021R 5 mg/kg (G2), traditional diuretics + clonidine 0.2 (G3), 0.5 (G4), or 1 mcg (G5), traditional diuretics + SSP-002021R 2 (G6), 7 (G7), or 10 mg/kg (G8).

Results: Natriuresis was higher in groups G1 (clonidine alone), G3 (diuretics + clonidine 0.2 mcg) and G7 (diuretics + guanfacine prodrug 7 mg/kg) than in the cirrhotic group treated with diuretics alone (G10) (all P < 0.03). Glomerular filtration rate (GFR) and renal plasma flow were higher in cirrhotic rats receiving clonidine (G1) or guanfacine prodrug (G2) than in cirrhotic rats receiving diuretics (G10) (all P < 0.03). The addition of guanfacine prodrug (2 mg/kg) in G6 to diuretics alone (G10) reduced tubular free-water reabsorption from 45 ± 12 to 20 ± 8 µL/min (aquaretic effect of α2-agonists) (P < 0.01), serum norepinephrine levels from 423 ± 122 to 211 ± 111 ng/L (P < 0.01) and plasma renin activity from 25 ± 12 to 9 ± 7 ng/mL/h (P < 0.01).

Conclusions: α2-agonists reduce adrenergic function and hyper-reninism and improve natriuresis in cirrhotic ascites, even when associated with common diuretics. The aquaretic effects of α2 agonists underline beneficial effects exerted on renal hemodynamics and GFR.

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VALUE-BASED OUTCOME INDICATORS IN THE MANAGEMENT OF HEPATOCellular CARCINOMA: CLINICAL TRIAL IN A LARGE MULTICENTER STUDY (VBMH STUDY)

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Introduction: The management of hepatocellular carcinoma (HCC) is a well-known public health problem, accounting for increasing mortality and high costs. Quality parameters able to measure clinical outcomes are still lacking.

Aim of our study (Value Based Medicine in Hepatology, VBMH) was to identify and test outcome indicators (OIs) in HCC, in light of their potential use in policy decision models.

Methods: A panel of experts identified a list of OIs using a modified Delphi method, according to experience and published evidence; four OIs with the highest RAND/UCLA score were tested in a prospective multicenter observational study. During 18 months, 711 HCC patients were enrolled and prospectively followed. 677 patients (95%) had at least one follow-up consultation. Median follow-up time was 14 months.

Results: The first OI was 1–3–5 years survival stratified for BCLC stage or treatment (OI#1). One-year survival for BCLC stage 0/A–D was 93%, 86%, 50%, 26% respectively. One-year survival of 288 patients treated for the first time during the study was 88% for liver transplantation, 97% surgical resection, 100% ablation and 89% for TACE. The other three OIs evaluated the appropriateness of treatments: worsening of BCLC and/or CPT score after three months from a loco-regional therapy or surgical resection (OI#2), occurred in 16% of cases (76% after TACE). Recurrence of HCC within 6 months after curative treatments (OI#3) happened in 15% of patients treated, mainly after ablation (20%). Presence of severe morbidity three months after loco-regional procedure or resection, evaluated as grade ≥ 3 according to Clavien-Dindo classification (OI#3), was found in 3% of patients treated.

Conclusions: The outcome indicators identified in the VBMH study proved their feasibility in a large cohort of patients and could stand as a benchmark for healthcare providers to move towards a value-based approach in the management of HCC.

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THE CHANGING SCENARIO OF HEPATOCellular CARCINOMA IN ITALY: AN UPDATE

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Background: Hepatocellular carcinoma (HCC) is the leading death cause amongst cirrhotic patients.

Aim: To evaluate the changes occurred in the last 12 years in clinical features, management and survival of HCC patients.

Methods: We analyzed the ITA.LI.CA (Italian Liver Cancer) database, updated at December 2012, including 3658 HCC patients at the time of tumour diagnosis. They were divided into three groups, according to the year of diagnosis: 2001–2004 (954 patients), 2005–2008 (1122 patients), 2009–2012 (1582 patients).

Results: The over time statistically significant (p < 0.01 or less) changes were: (1) mean age progressively increased; (2) liver function (Child-Pugh class) improved; (3) prevalence of HCV infection decreased, while non alcoholic steatohepatitis (NASH) and cryptogenic cirrhosis increased; (4) patients with normal alpha-fetoprotein (<10 ng/ml) increased; (5) diagnosis under surveillance remained stable (but with an increasing proportion of surveillance intervals ≤6 months) while symptomatic diagnosis increased; (6) BCLC early stages increased; (7) proportion of patients treated with radiofrequency increased, at the expense of percutaneous ethanol injection, chemoembolization and liver transplantation; (8) 1- and 3-year survival rates progressively increased.

Conclusions: Diagnosis, treatment and prognosis of HCC are continuously changing. Survival increase may be related to improved management of cirrhosis, earlier tumour-stage and better liver function at diagnosis. Diagnosis during surveillance did not increase, principally due to an inconsistent application of surveillance, but also to the increasing prevalence of HCC on NASH/cryptogenic cirrhotic patients - ordinary lacking a surveillance programme. This should prompt new guidelines for surveillance of patients at risk.

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**F-44**

**SORAFENIB VERSUS \(^{90}\)Y-90-RADIOEMBOLIZATION: A PRELIMINARY ASSESSMENT OF TOLERABILITY AND SURVIVAL IN ADVANCED MONO-LOBAR HEPATOCELLULAR CARCINOMA**

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**Introduction:** Sorafenib and \(^{90}\)Y-90-Radioembolization (TARE) are the optional therapies for cirrhotic patients with advanced mono-lobar hepatocellular carcinoma (HCC).

**Aim:** To evaluate survival and tolerability of therapy with sorafenib or TARE in cirrhotic patients with advanced mono-lobar HCC.

**Materials and methods:** Data from our prospective database were analyzed. Inclusion criteria: Child-Pugh A liver cirrhosis with mono-lobar HCC; BCLC stage B or C; M0; NO/N1; treatment with sorafenib for more than 60 days or with TARE (1–2 sessions); follow-up longer than 180 days. Liver-related complications, side effects and survival (Kaplan–Mayer curves) were analyzed.

**Results:** Between January 2008 and November 2013, 177 patients received sorafenib and 33 were included: 29 male; median age: 69 years (range: 32–82); ECOG 0; median tolerated drug dose: 800 mg (200–800); median treatment time: 4.9 months (range: 2–23.4). Between May 2011 and November 2013, 38 patients underwent TARE and 30 were included: 29 male; median age: 66.5 years (range: 50–79); ECOG 0; treated with 1 (29/30) or 2 sessions. Side effects occurred in 94% (31/33) of patients treated with sorafenib (mostly asthenia, hand-foot skin syndrome, diarrhea, nausea) and in 27% (8/30) of patients treated with TARE (nausea, vomiting, allergic reaction to contrast media) \((p < 0.001)\). Liver function worsened (Child-Pugh \(\geq B\) in 24% (8/33) of sorafenib and in 10% (3/30) of TARE group after 30–60 days from therapy \((p < 0.05)\).

Overall 44 deaths occurred (27 sorafenib, 7 TARE). Median overall survival was 11 months (95% CI: 9.6–12.4) with sorafenib and 12 months (95% CI: 9.0–15.1) with TARE \((p > 0.05)\). Cumulative probability of survival at 6–12–18 months was 84.3–40.6–19.0% with sorafenib versus 86.3–48.2–33.0% with TARE \((p > 0.05)\).

**Conclusions:** TARE has a lower rate of side effects but similar overall survival compared to sorafenib in cirrhotic patients with advanced mono-lobar HCC.

**F-45**

**FIRST-LINE TREATMENT OF 90 CONSECUTIVE HCC WITH DRUG ELUTING BEADS CHEMOEMBOLIZATION (DEB-TACE)**

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**Background/aims:** Aim was to evaluate the application of drug eluting beads chemoembolization (DEB-TACE) as first-line treatment of HCC in a consecutive series of cirrhotic patients discussed by a multidisciplinary clinic (MDC) team in a single tertiary centre.

**Methods:** Out of 383 consecutive patients with a de-novo HCC observed between January 2007 and December 2011, 90 (23%) not suitable for liver transplantation were proposed for DEB-TACE: 65 (72%) males, mean age 68 (range 47–76), 63 (70%) Child-Pugh A, 44 (49%) BCLC A, 37 (41%) BCLC B, 9 (10%) BCLC C. DEB-TACE was repeated every two months until complete response or progression, assessed by CT-scan, according to modified RECIST criteria. Recurrent HCCs and progressive disease were further treated according to MDC decision. Discrepancies to AASLD/EASL-EORTC recommendations were recorded.

**Results:** During 41 months (range 6–142), 30 (33%) patients died. Complete response was reported in 19 (21%). Overall yearly mortality rate was 12%, corresponding to 1, 3, 5 yr survival 92%, 81%, 57%. These figures were 6%, 98%, 84%, 71% in BCLC A; 17%, 94%, 54%, 42% in BCLC B; 57%, 60%, 14%, 0% in BCLC C, respectively. Independent predictors of survival were number of nodules HR 1.14 (95% CI 1.1–1.2, \(p < 0.001\)), AFP > 200 ng/mL HR 1.95 (95% CI 1.5–2.5, \(p < 0.001\)) and ascites HR 2.16 (95% C.I. 1.1–3.4, \(p = 0.001\)).

**Conclusions:** First-line treatment with DEB-TACE achieved 71% 5 years survival in BCLC A, comparable to radical therapies and it can be safely applied to advanced HCCs with peripheral neoplastic portal thrombosis without extrahepatic spread.

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**F-46**

**PHARMACOKINETICS OF SUNITINIB IN PATIENTS WITH LIVER CIRRHOSIS AND HEPATOCELLULAR CARCINOMA**

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**Introduction:** Sunitinib is a multitargeted tyrosine kinase inhibitor currently employed in renal-cell carcinoma, gastrointestinal stromal tumour, pancreatic neuroendocrine tumors. Sunitinib is metabolized by hepatic CYP3A4 and biliary excreted, but pharmacokinetic in patients with liver cirrhosis is poorly known. Aim of the study is to characterize the pharmacokinetics of sunitinib in cirrhotic patients with hepatocellular carcinoma (HCC).

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Methods: 15 cirrhotic patients (mean age 68.2 years, 13 male, 11 Child-Pugh A, 4 Child-Pugh B) enrolled in a phase II study on HCC (Dig Liver Dis 2013;45:692–8) received sunitinib 50 mg daily on a 4-week-on-2-week-off schedule. Blood samples were collected at 0,1,2,4,8,12,18, and 24 hours after the first oral dose and at 3rd, 4th and 28th days later. Drug plasma concentrations were measured by a validated HPLC method. Non-compartmental Statistical Moment Theory was used for pharmacokinetic parameters.

Results: Sunitinib maximum concentration (C_{max}) of 82.4 ± 81.8 ng/mL were observed after 12.9 ± 8.1 h (T_max). Area Under the Curve (AUC_{0-24}) was equal to 1128.5 ± 985.6 ng h/mL and Mean Residence Time (MRT) equal to 59.7 ± 43.4 h, markedly higher than values reported for patients with healthy liver (J Clin Oncol 2006;24:25–35). 9/15 patients discontinued sunitinib for adverse events, but AUC_{0-24} or MRT were not statistically different compared with patients that stopped drug for progression. Among liver function tests, pre-treatment alkaline phosphatase was linearly related to AUC_{0-24} (r^2 = 0.33) and inversely related to MRT (r^2 = 0.26).

Conclusions: Pharmacokinetics of sunitinib in patients with cirrhosis differ markedly from data published for patients with healthy liver, so it is plausible to assume an individualized dose in cirrhotic patients.

Pharmacokinetic parameters of sunitinib in patients with liver cirrhosis (n = 15)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (ng/mL)</td>
<td>82.4 ± 81.8</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>12.9 ± 8.1</td>
</tr>
<tr>
<td>AUC_{0-24} (ng h/mL)</td>
<td>1128.5 ± 985.6</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>59.7 ± 43.4</td>
</tr>
<tr>
<td>T_{1/2} (h)</td>
<td>41.6 ± 31.1</td>
</tr>
<tr>
<td>C_{trough} day 28 (ng/mL)</td>
<td>62.6 ± 52.4</td>
</tr>
</tbody>
</table>

Mean ± Standard deviation. C_{max} maximum concentration; T_{max} time to C_{max}; AUC_{0-24} area under the concentration–time 0–24 h; MRT Mean Residence Time; T_{1/2} half-life; C_{trough} trough concentration.

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F-47

LEYKAEMIA INHIBITORY FACTOR PROTECTS CHOLANGIOCARCINOMA CELLS FROM CYTOTOXICITY VIA A STAT-3-INDEPENDENT, MCL-1 ACTIVATION

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Introduction and aim: Cholangiocarcinoma (CCA), an aggressive liver malignancy with strong chemo-resistance, is surrounded by an abundant tumor reactive stroma, where cytokines regulating cell proliferation and survival are released. Their modulation may represent a potential therapeutic target. Among these cytokines, IL-6 is a potent growth factor for normal and neoplastic cholangiocytes. We sought to investigate the effects of leukaemia inhibitory factor (LIF), a member of the IL-6 family, that induces proliferation in several cancers, but has unknown effects in CCA.

Methods: LIF and LIF-receptor (LIFR) expression was evaluated in specimens of CCA and peri-tumoral areas (immunohistochemistry) (n = 10), and in established (HuCCT-1, TFK-1, EGI-1) and primary (n = 8) human CCA cell lines obtained from resected livers (ELISA, Western blotting, WB). Cell proliferation, cell invasion (Boyden chambers) and cell viability of TFK-1 and HuCCT-1 (MTS) were evaluated in response to recombinant human (rh) LIF (0.1-100 ng/mL) with/without different chemotherapeutics (Paclitaxel, Camptothecin, Platinum, Gemcitabine). In LIFR-expressing cell lines (TFK-1 and HuCCT-1), Bax (pro-apoptotic), Mcl-1, Bcl-2 (anti-apoptotic), and STAT-3 (IL-6 downstream effector) were analyzed by WB upon rhLIF stimulation (10, 100 ng/mL).

Results: In tissue sections and cultured cells, LIF and LIFR expression was restricted to neoplastic cholangiocytes; LIF had negligible effects on cell proliferation and invasion, but significantly counteracted (by around 25%) drug-induced apoptosis. In LIF-treated cells, expression of Mcl-1 increased significantly, whilst Bax and pSTAT-3 remained unchanged. Bcl-2 was not expressed in CCA cells.

Conclusions: Autocrine and paracrine LIF-dependent mechanisms may contribute to CCA progression by providing neoplastic cholangiocytes with pro-survival capabilities. LIF signalling involves STAT3-independent expression of Mcl-1. This effect may be relevant to increase CCA chemoresistance.

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F-48

LYSOSOMES INVOLVEMENT IN CHEMORESISTANCE OF HEPATOCELLULAR CARCINOMA CELLS

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Background and aims: Multi-drug resistance proteins (MDRPs) play an important role in chemoresistance of cancers, including hepatocellular carcinoma (HCC). We observed that Sunitinib, a yellow antineoplastic agent, accumulates in cytoplasmic vesicles of cultured HCC cell lines; therefore, we investigated the relationship of vesicles with MDRPs and chemoresistance of cultured HCC cells to Sorafenib, currently used in the treatment of advanced HCC, and the presence of these vesicles in primary HCC samples.

Methods: Cytotoxic effect of Sorafenib alone or in combination with Verapamil, an inhibitor of MDRPs, was tested by MTT on HuH7, HepG2, Hep3B, PLC/PRF/5, SNU475 and hcc-1. Immunofluorescence (IF) staining (PDI for endoplasmic reticulum, PMP70 for peroxisomes, LAMP-1 for lysosomes and VMP1 for autophagosomes) was performed to identify vesicle origin and cell localization of MDRPs (ABCB1, ABCC1, ABCG2 and MVP). Immunohistochemistry (IHC) for autophagy proteins was performed on 4 cirrhosis/HCC human samples.

Results: IF showed that only ABCB1 and LAMP-1 were overexpressed on vesicle. Cell lines could be classified in two groups: (1) lines with small multiple vesicles visible only after drug treatment (Hep3B, PLC/PRF/5 and SNU4755) and (2) lines with medium-large...
vesicles already visible at basal condition (HepG2, hcc-1 and HuH7). Interestingly, group 1 cells were less resistant to 15 μM Sorafenib (viability: 19% Hep3B, 45% PLC/PRF/5 and 45% SNU475) than group 2 (viability: 95% HepG2, 68% hcc-1 and 82% HuH7). Verapamil treatment increased the efficacy of Sorafenib in all cultures, especially if added after Sorafenib. IH showed PGP canalicular expression in cirrhosis, while in 1 of the four HCC samples we observed cyttoplasmic vesicles staining.

Discussion: Our data showed that HCC cells segregate anti-neoplastic drugs in lysosomes expressing ABCB1. A larger size of lysosomes was associated with chemoresistance in HCC cell lines. It is possible to observe lysosomes expressing ABCB1 also in some primary HCC samples.

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F-49

PAF-RECEPTOR ANTAGONISTS REDUCE HCC PROGRESSION BLOCKING CANCER CELL PROLIFERATION AND MIGRATION

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Introduction: Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. However, there is no effective treatment for advance stage HCC. PAF-receptor antagonists WEB-2086 and WEB-2170 (WEBs) have been previously shown to induce differentiation, growth arrest and massive apoptosis in murine and human leukemia cells.

Aim: We investigate the anti-tumor efficacy of WEBs in vitro and in vivo models.

Materials and methods: We evaluated proliferation, apoptosis and migration of different hepatic cancer cell lines (HepG2, Hep3B, HuH7 and Hepa1-6) incubated with WEBs. We tested the in vivo efficacy of the treatment in HBV transgenic mice (TgAlb43Bri), that spontaneously develop hepatic tumors, and in a syngeneic orthotopic murine HCC model, where HCC cells were implanted in directly in the liver.

Results: WEBs were able to reduce the proliferation of cancer cells as assessed by thymidine incorporation, but no notable effects were present on apoptosis as assessed by caspase 3 activity. Furthermore pre-incubating the hepatic cancer cells with the WEBs induced cell cycle arrest and impaired migration in a wound healing assay. Treatment with WEB reduced growth and reduced proliferation of the tumors in the transgenic and in the orthotopic murine model.

Conclusions: PAF-receptor antagonists, WEB-2086 and WEB-2170, are able to reduce HCC progression in human and murine HCC models blocking cancer cell proliferation and migration.

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F-50

MICRONVASIVE INTRAOPERATIVE ULTRASOUND PATTERN IN PREDICTING OUTCOMES FOR SINGLE SMALL (<3 CM) HEPATOCELLULAR CARCINOMA

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Background: The significance of tumor microinvasion (portal venous, hepatic vein, or bile duct infiltration and/or intra-hepatic metastasis, MI) in patients with single, small (≤3 cm) hepatocellular carcinoma (HCC) remains unclear.

Aim of the study was to evaluate MI impact (MI+ vs. MI−) on HCC recurrence and long-term survival in patients treated by hepatic resection (HR) or surgical radiofrequency ablation (RFA).

Methods: All patients with single, small HCC who underwent HR or RFA between 1997 and 2013 were included in this study. In agreement with the histological criteria described by Yamashita,1 the intraoperative ultrasound (IOUS) definition of MI has been defined according to our previous report on IOUS HCC classification.2

Results: 370 patients (141 HR) were included: 144 (39%) patients had MI+: 33% in VE-HCC (≤2 cm) and 49% in the others (2–3 cm) (p = 0.002). In the HR subgroup, IOUS findings were associated with histopathologic features (p = 0.0001), sensitivity to detect MI+ being 84% and positive predictive value being 86%. MI+ patients had significantly worse survival than MI− (5-years survival: 33% vs. 49%, respectively; p = 0.0358) and higher intra-hepatic recurrences (5-years HCC recurrences: 86% vs. 64%, respectively; p = 0.0001). No difference was found among MI+ patients submitted to HR or RFA for long-term survival or intra-hepatic recurrence, while a higher rate of local recurrence was found after RFA (5-year local recurrences: 48% vs. 9%, respectively; p = 0.0001).

Conclusion: In patients with single, small HCC, the prevalence of MI+ is high, even in cases of HCC ≤2 cm. IOUS findings strongly correlated with histopathologic criteria in detecting MI+. MI+ HCC patients had a worse prognosis, irrespective to treatment. IOUS findings may be used, as valuable tool, to determine the intraoperative choice of treatment and to predict the patients’ prognosis.

References

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IMMUNO-MODULATING EFFECTS OF SILIBININ IN LIVER TRANSPLANT PATIENTS WITH HCV RECURRENTS

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Background and aim: Silibinin (Sil) has been proven to have anti-viral activity in humans and it has been successfully used in our center for HCV recurrence treatment in liver transplant patients. Sil modulates dendritic cells (DC) function. DC are antigen-presenting cells playing, along with regulatory T cells (Treg), a pivotal role in controlling allo-immune response, as well as HCV infection. Immune regulatory molecules expressed by DCs, including PD-L1, ICOS-L, CD39, HLA-G, and the ILT4, have been shown to regulate T cell responses, including the induction of Treg. The PD-1/PD-L1 pathway on Treg modulates HCV T cell responses. Aim of the study is to analyze circulating DC subsets and Treg and the expression of costimulatory/coregulatory molecules in liver transplant patients receiving Sil.

Material and methods: 15 liver transplant patients with HCV recurrence received iv infusion of Sil (20 mg/kg/day) for 14 consecutive days. We examined by flow cytometry, before and at the end of treatment, the expression of CD83, CD86, PD-L1, ICOS-L, CD39, HLA-DR, HLA-G, IL-T4 in circulating monocytoid (m) and plasmacytoid (p) DC and of PD-1 on Treg.

Results: After Sil treatment we observed a higher pDC/mDC ratio and pDC exhibited a lower HLA-DR and a higher IL-T4, CD39 and HLA-G expression as compared with the baseline. No correlation was found between these markers and HCV viral load. In addition, after Sil treatment mDC show a higher ICOSL expression that was inversely correlated to viral load. No changes were detected in Treg frequency and PD-1 expression.

Conclusions: This is the first study in liver transplant patients with HCV recurrence showing the impact of Sil on DC and Treg. Findings show changes, not correlated with viral load, in circulating pDC that have previously been associated with tolerogenic conditions, providing new insight into how Sil might regulate alloimmunity.

Reference

Angiogenesis in Chronic Hepatitis C Recurring After Liver Transplantation is Correlated with Splanchnic Hemodynamics

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Background/aims: In chronic hepatitis (CH), both angiogenesis and fibrogenesis are key determinants of disease progression and portal hypertension development. Despite this, the histological assessment of CH has always neglected vascular changes, providing greater attention to the extent of fibrosis. This study is aimed to histologically evaluate angiogenesis and its correlations with splanchnic hemodynamics in a series of post-transplant relapsing chronic hepatitis C (RCHC).

Methods: Angiogenesis was immunohistochemically assessed (anti-CD34 monoclonal antibody) in 40 liver biopsies of post-transplant RCHC. Portal tract microvessel density (MVD) was scored on a scale from 0 to 3; sinusoid capillarization (SC) was scored as absent, peri-portal (CD34-positive sinusoids around portal tracts), and diffuse (CD34-positivity extending into the lobular parenchyma). Grading (HAI) and staging of CH were performed according to Ishak’s system. Hemodynamic parameters (portal blood flow velocity [PBV], hepatic artery pulsatility index [HPI], splenic PI [SPI] and portal hypertension index [PHI]) were evaluated at the time of liver biopsy by echo-color Doppler, as previously described. Cases with rejection or any vascular alteration which may affect splanchnic Doppler parameters were excluded.

Results: Early or intermediate stage of fibrosis (score 1–4) were documented in 95% of cases. Moderate to high MVD was found in 47.5% of cases and was significantly associated with higher fibrosis and HAI scores. A trend toward an association between higher MVD and reduced PBV was also observed. SC was detected in 57.5% of cases (score 1: 13 cases; score 2: 10 cases) and significantly correlated with reduced PBV (p = 0.0001) and higher HPI values (p = 0.0007).

Conclusions: This is the first observation that tissue angiogenesis reflects hemodynamic changes. Its assessment in liver biopsies may provide prognostic information and should become part of the histological assessment of liver biopsies in course of chronic liver diseases.

Reference
http://dx.doi.org/10.1016/j.jdl.2014.01.150

EFFECT OF PORTAL VEIN THROMBOSIS (PVT) ON SURVIVAL AFTER LIVER TRANSPLANTATION (LT): A META-ANALYSIS


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Background: PVT is a common complication in patients with liver cirrhosis undergoing LT. Although PVT is no longer considered an absolute contraindication to LT, published data of its effect on mortality after the surgery are heterogeneous and discordant. The aim of the present study was to systematically review the current literature on the role of PVT in LT recipients in term of outcome.

Methods: A systematic review of the English and non English literature was performed by analyzing studies that report on PVT in LT recipients and were published between January 1986 and January 2013. We performed a meta-analysis using the 30-day and 1-year mortality as endpoints in all the studies, using random effect model.

Results: Twenty six studies among the total of 426 articles initially retrieved were considered. Of 25,753 LT, 2004 were performed in patients with PVT (7.8%), and approximately half had complete thrombosis (according to Yerdel’s classification) at the time of LT. Seven studies report on 30-day mortality in both patients with PVT and without PVT, for a pooled mortality rate of 10.5% vs. 7.7% (P = 0.01) respectively with OR equal to 2.29 (95% CI...
USEFULNESS OF ACOUSTIC RADIATION FORCE IMPULSE (ARFI) IN THE ASSESSMENT OF LIVER FIBROSIS DUE TO RECURRENT HEPATITIS C AFTER LIVER TRANSPLANTATION

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Background and aims: Acoustic radiation force impulse (ARFI) demonstrated high accuracy in the non invasive assessment of liver fibrosis in HCV chronic hepatitis and in HCV positive pediatric recipients. The study aimed to assess the accuracy of ARFI and APRI test in detecting liver fibrosis in HCV positive adult recipients.

Methods: Forty three consecutive HCV positive recipients (34 males, mean age 62 years) were prospectively enrolled. At a median time of 6 years following LT, annual liver biopsy (median 9 portal tracts), ARFI measurement in the right and left lobe of the graft and APRI calculation were performed in the same day. ARFI was performed ten times for each recipient and was expressed as mean (±SD) m/sec. Median (IQR) Ishak staging score was 1 (0–2). In detail 28 (65%) recipients had Ishak staging 0–1, 12 (28%) had 2–3 and 3 (7%) had 4–5. Mean (±SD) ARFI shear wave velocity was: 1.57 (±0.29) m/sec in the right lobe (FD) and 2.06 (±0.40) in the left lobe (FS). Mean (±SD) APRI was 0.88 (±1.02). Mean (±SD) of FS ARFI were 1.85 (±0.53), 2.38 (±0.59) and 2.85 (±0.43) for Ishak staging 0–1, 2–3 and 4–5 respectively (p < 0.002). The corresponding APRI means were 0.64 (±0.56), 1.27 (±1.58) and 1.52 (±1.21, p = 0.108). AUROC ARFI in discriminating Ishak staging score > 2 was 0.874 (p < 0.001); using a cut-off level of 2.34 m/sec sensitivity, specificity and accuracy were 100%, 78.4% and 78.4%, respectively.

ARFI could be considered a useful non invasive method for monitoring liver fibrosis in HCV positive recipients.

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THE INFLAMMASOME PATHWAY AS A TARGET OF BERBERINE IN EXPERIMENTAL STEATOHEPATITIS AND ACETAMINOPHEN TOXICITY

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Background/aims: Berberine (BRB), an alkaloid has been shown to have hepatoprotective properties, but the underlying molecular mechanisms are poorly understood. Aim of this study was to explore the anti-inflammatory mechanism of action of BRB in murine models of acute and chronic liver injury.

Materials/methods: BRB was tested in steatohepatitis induced by administration of a methionine and choline deficient (MCD) diet, and in acute acetaminophen (APAP) intoxication. LPS-stimulated murine macrophages (RAW264.7) were employed as an in vitro model.

Results: BRB markedly ameliorated liver injury and inflammation induced by the MCD diet, and downregulated intrahepatic expression of proinflammatory genes. BRB limited Expression of all components of the NALP3 inflammasome pathway and hepatic levels of mature IL-1beta increased in animals fed the MCD diet and were reduced by BRB. In acute liver injury caused by APAP overdose, BRB reduced mortality, limited ALT elevation and modulated the upregulation of inflammasome components. No effects on glutathione levels were found at 6 h after APAP intoxication. Inflammasome activation in LPS-stimulated RAW264.7 cells was markedly decreased by BRB, demonstrating a direct interference with activation of the inflammasome pathway. BRB did not significantly affect the ‘first signal’ of the inflammasome pathway, while it reduced the elevation of intracellular calcium caused by a selective ligand of P2X7, a purinergic receptor implicated in the generation of the ‘second signal’.

Conclusions: BRB ameliorates inflammation and damage in two models of experimental liver injury. We also demonstrate for the first time that BRB interferes with activation of P2X7, a purinergic receptor involved in inflammasome activation.

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Abstracts of the 47th A.I.S.F. – Italian Association for the Study of the Liver – Annual Meeting 2014
Abstracts Evaluation Procedure

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