Contribution of visceral fat and hepatic fat to metabolic derangements and liver damage in NAFLD patients.

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OC-07
Primary biliary cirrhosis and metabolic syndrome
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Background: PBC is characterized by a long natural history and a low incidence of cardiovascular events despite high cholesterol serum levels. However, the role of metabolic conditions (obesity, hypertension, insulin resistance) eventually associated to PBC has not been analyzed.

Aim: To assess the influence of metabolic syndrome (MS) on the response to UDCA and the survival in PBC patients.

Methods: The historic database collection (1975–2011) was used. The mean follow up was 123 months, (range 6–425 months). All patients were treated with UDCA (15 mg/kg/day). Hypercholesterolemia was treated with statins or fibrates when total cholesterol was >240 mg/dl. Responders to UDCA were defined subjects who reached at least a 40% decrease in alkaline phosphatase after 1 year. MS was defined MS according to the American Heart Association criteria. Survival was analyzed with Kaplan-Meier curves.

Results: A total of 171 PBC patients were considered eligible for the study; 55 of them (32.1%) at presentation fulfilled the criteria for MS. LFTs and Mayo score were comparable in patients with MS and in those without MS; only GGT resulted significantly higher in patients with MS (243 vs. 159 IU/ml, p=0.038). Histological stages and fibrosis score were similar in the two groups at baseline. The occurrence of cardiovascular events during the follow-up was significantly higher in patients with MS (p<0.01). The response to UDCA was higher in the group without metabolic syndrome (p=0.05). Kaplan-Meier curves were similar until a 200 month interval, thereafter survival was worse in the group with MS, but the difference was not statistically significant.

Conclusion: MS when associated to PBC should be carefully treated, due to the risk of cardiovascular events and the reduced response to UDCA.

OC-08
Dysbiosis contributes to liver fibrosis through bacterial translocation and elevated endotoxinemia by increasing Gram– abundance
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Introduction: Non-alcoholic fatty liver disease may lead to hepatic fibrosis. Dietary habits affect gut microbiota; while endotoxins produced by Gram– bacteria stimulate hepatic fibrogenesis. However, the mechanisms involved in the process of liver damage and the potential effect of microbiota is still unknown.

Aim: To analyze whether microbiota composition and bacterial translocation may interfere with liver fibrogenesis.

Material, methods, and results: Mice were treated with control (CTRL) or high fat (HFD) diet for 1 month and subsequently subjected to either BDL (2 weeks) or i.p. CCI4 (3 weeks). Microbiota transplantation was obtained by oral gavage of previously gut-sterilized mice by antibiotic cocktail (Neomycin, Ampicillin, metronidazole, vancomycin). mRNA for collagen α1(I) and αSMA, collagen deposition (Sirius Red), hydroxyproline content, were increased in the HFD-BDL mice versus CTRL-BDL mice, while no differences were observed between CTRL-CCI4 mice and HFD-CCI4 mice. Culture of mesenteric lymphnodes showed higher density of infection in HFD-BDL mice versus CTRL-BDL mice, suggesting higher bacterial translocation rate. No evidence of bacterial translocation was observed in CCI4-treated mice. HFD-BDL mice showed the highest reduction in occludin mRNA expression, suggesting increased intestinal permeability. Pyrosequence analysis revealed a condition of dysbiosis characterized by an increase of Gram+ bacteria, a reduced ratio between Bacteroidetes and Firmicutes and, more specifically, a dramatic increase of the Gram− proteobacteria in HFD-BDL versus CTRL-BDL mice. Inflammmasome expression was increased in fibrotic liver, but significantly reduced in the gut. Furthermore, microbiota transplantation revealed more liver damage in the chimeric mice treated with CTRL diet but receiving the microbiota of HFD-treated mice.

Conclusions: By increasing the percentage of Gram− producing endotoxins, different dietary habits lead to a pro-fibrogenic effect in the course of chronic hepatic liver damage. Bacterial translocation, altered intestinal permeability and dysbiosis are needed by hepatic steatosis to act as co-factors in chronic liver diseases.

OC-09
Contribution of visceral fat and hepatic fat to metabolic derangements and liver damage in NAFLD patients
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Background: Both hepatic (HF) and visceral fat (VF) are involved in the onset and progression of metabolic syndrome and NAFLD. However, their relative role is still debated.

Aim: To determine the relative contribution of HF and VF to both metabolic derangements and histological liver damage in NAFLD patients.

Methods: In 22 non-diabetic, non-dyslipidaemic patients with biopsy-proven NAFLD we measured whole abdomen VF by MRI, fasting endogenous glucose production (EGP) and lipolysis by tracer infusion, peripheral insulin resistance (IR) as HOMA, Hepatic-IR (EGP*insulin) and Adipo-IR (FFA*Insulin). Hepatic histology was scored according to Kleiner, HF was assessed as percentage of liver fat.

Results: VF increased with BMI (r=0.54, p<0.004) and HF (r=0.43, p<0.03),...
Hepatocyte-derived microparticles released in blood as biomarkers of NASH development

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Introduction and aim: At present there is a lack of effective treatments and noninvasive diagnostic markers for nonalcoholic steatohepatitis (NASH). We have recently demonstrated that hepatocyte-derived microparticles (MPs) are critical signals contributing to angiogenesis and liver damage in NASH (Presidential Plenary, AASLD 2012). Here we tested the hypothesis that circulating hepatocyte-MPs are novel targets for noninvasive NASH monitoring.

Methods: Male C57BL/6 mice were placed on Choline-Deficient L-Amino Acid (CDAA) diet, Choline-Supplemented L-Amino Acid (CSAA) or regular diet (RD) for 4 and 20 weeks (early stage and established NASH, respectively). Circulating MPs were isolated from platelet-free plasma, detected by flow cytomtery and extensively characterized by electron microscopy in liver tissue and circulation, dynamic light scattering and by LC MS/MS proteomic analysis. Liver specimens were collected and used for histological, biochemical, and molecular analysis of steatosis, inflammation, angiogenesis, fibrosis and cell death.

Results: MPs circulating levels were significantly increased only in mice with established NASH (20 wks CDAA diet: 304,400 MPs/mL vs. 20 wks CSAA 34,300 MP/mL vs. RD 2,000 MP/mL, p < 0.0052). The increase was time-dependent and MPs blood levels strongly correlated with histological features of liver damage, in particular with fibrosis, as determined by morphologic quantification of Sirius Red staining (r=0.736; p<0.0002), and cell death determined by TUNEL assay (t=0.804; p<0.0001). We next characterized the antigenic composition of circulating MPs using a comprehensive proteomic approach by LC-MS/MS analysis. A gene ontology analysis of the proteins identified 26.5% plasma membrane proteins, 58.8% cytoplasmic proteins, 8.8% nuclear proteins and 5.9% extracellular proteins. Analysis of the molecular function of these proteins demonstrated that 35.3% were enzymes, 41.2% were cytoskeleton or vesiculation proteins, 29.0% nucleosome proteins and 5.9% ribomacleoproteins.

Conclusion: Our data identified circulating MPs with a unique antigenic composition as potential novel biomarkers for noninvasive diagnosis of NASH.

PNPLA3 GG genotype is associated with carotid atherosclerosis in patients with non-alcoholic fatty liver disease

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Background and Aims: There is evidence that patients with non-alcoholic fatty liver disease (NASH) are at high cardiovascular risk and that the prevalence and the severity of NASH are also driven by genetic background. In NASH patients we tested the association of carotid atherosclerosis with clinical, genetic and histological factors.

Materials and Methods: We assessed anthropometric, metabolic and histologic data (Kleiner score) in 162 consecutive biopsy-proven NASH patients. Intima-media thickness (IMT) and carotid plaques, defined as focal thickening of >1.3 mm at the level of common carotid artery, were evaluated using ultrasonography. IL28B rs12979860 C>T, PNPLA3 rs738409 C>G, GCKR rs780094 C>T, LYPAL1 rs12137855 C>T, and NCAN rs2228603 C>T single nucleotide polymorphisms were also assessed.

Results: Carotid plaques were found in 59 (36.4%) cases, and in particular, in 32% PNPLA3 CC/CG vs. 52.9% PNPLA3 GG patients (p=0.02). Multivariate logistic regression analysis showed that older age (OR 1.102), female gender (OR 2.476), type 2 diabetes (OR 4.061), and PNPLA3 GG genotype (OR 2.679) were independently linked to the presence of carotid plaques. In patients aged <50 years, 877 cases with PNPLA3 GG genotype (10.9%) had carotid plaques, vs. 615 (40%) with PNPLA3 GG genotype (p=0.02). By contrast, in patients ≥50 years the prevalence of carotid plaques was similar in subjects with PNPLA3 CC/CG and GG genotype (33/55, 60% vs. 12/19, 63%; p=0.75). Similarly, PNPLA3 CC/CG cases aged <50 had a lower IMT than PNPLA3 GG patients (0.75±0.17 vs. 0.85±0.25 mm; p=0.05), while this difference was not observed in patients aged ≥50 (0.93±0.25 vs. 1.00±0.26 mm; p=0.32).

Conclusion: PNPLA3 GG genotype is associated with a high risk of early carotid atherosclerosis in NASH.

P53 and hepatic lipid metabolism: a new interesting connection

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease worldwide, ranging from simple steatosis to steatohepatitis which may progress to cirrhosis, and hepatocellular carcinoma. However, the molecular mechanisms underlying NAFLD development have not been completely characterized. Recently, the p53 tumor suppressor protein has gained attention as a modulator of cellular metabolism, responding to several stress conditions, but its role in the progression of NAFLD remains poorly understood. We designed an in vitro model of NAFLD to analyze how different forms of p53 may affect the metabolic stress response induced by free fatty acids (FFAs) overloading.

Materials and methods: Three cell lines carrying different forms of p53: HepG2 (p53 wt), Huh7.5.1 (mutated-p53) and Hep3B (p53 knockout), were cultured for 14 h in a medium containing oleic and palmitic acid (molar ratio 2:1, respectively) at a final concentration of 1 mM, the most abundant FFAs in western diets. Intracellular lipid accumulation and cell viability were assessed by adipored and MTS assay, respectively. Gene and protein expression profiles of p53 and lipid metabolism-related genes were evaluated by qRT-PCR and Western blotting.

Results: In the three cell lines the FFA treatment was not cytotoxic and...