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SERUM PROTEOMICS PROFILING IN HCV PATIENTS WITH LIVER FIBROSIS. K.J. Cheung (1), D. Deforce (2), K. Tilleman (2), I. Colle (1), H. Van Vlierberghe (1). (1) University Hospital Ghent, Department of Hepatology and Gastroenterology ; (2) Ghent University, Laboratory of Pharmaceutical Biotechnology.

**Objective** : Liver biopsy for the assessment of liver fibrosis in HCV patients is an invasive, unpleasant and suboptimal method. Therefore the aim of this study was to discover and identify new potential biomarkers for liver fibrosis in HCV patients using proteomic technology.
**Methods** : A HCV patient cohort (n = 30) was selected and matched according to age (at biopsy), weight, alcohol consumption andduration of infection. The cohort was divided according to the 5 fibrosis stages (Metavir) ; F0 : none (n = 6) ; F1 : mild (n = 6) ; F2 : moderate (n = 6) ; F3 : advanced (n = 6) ; F4 : cirrhosis (n = 6). Serum samples were depleted from albumin and IgG and were used for theanalysis.The proteins were separated using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) (pH-gradient 4-7, 10% SDS-PAGE) and visualized with a fluorescent protein marker, Sypro Ruby (Molecular Probes). Analysis of the gels was performed using PD-Quest (Bio-Rad). The different gels were matched and regrouped to the 5 fibrosis stages (F0-F4) and inflammatory activities (A0-A3). The groups were analyzed with the Student's t-test and the Mann-Whitney U-test ($p < 0,05$). Proteins were identified using two complementary mass spectrometry (MS) techniques, namely MALDI-Q-TOF MS and ESI-Q-TOF MS/MS on-line coupled to a nano-Liquid Chromatography (LC) system.
**Results** : After gel analysis a total of 1356 separated spots have been matched between all gels. 117 protein spots were differently expressed of which 91 spots between different fibrosis stages (F0-F4) and 26 spots between inflammatory activities (A0-A4). Among these spots alpha-2-macroglobulin (F0 < F4), serum albumin (F0 > F1 ; F0 > F2), haptoglobin (F1 > F4) were identified, which are currently used in non-invasive alternatives.
**Conclusions** : During this discovery phase of our proteomics analysis, protein patterns have been identified that can discriminate different stages of liver fibrosis. Among the 117 differently expressed proteins, so-called surrogate markers of non-invasive alternatives have been identified. This indicates that our approach has potential to discover and identify biomarkers for different stages of liver fibrosis and posses possibly new unknown marker proteins. However the identified proteins need to be further investigated to characterize their involvement in the pathology and their potential as true fibrosis markers.

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AQUAPORIN-4 EXPRESSION IN RATS IN FULMINANT HEPATIC FAILURE. O. Detry, N. Meurisse, M. Meurisse, J. Defraigne, B. Rogister. CHU Liège.

**Background and aim** : The pathophysiology of brain oedema of fulminant hepatic failure (FHF) is not fully understood at the molecular level. Aquaporin-4 (AQP4) is a water channel membrane protein that eases the water movement across cell membranes. In the brain AQP4 is largely expressed in cortical astrocyte feet processes. AQP4 plays a role in various cytotoxic brain oedema, but was not investigated in FHF. We studied the expression of AQP-4 in a FHF rat model.
**Methods** : FHF was induced in rats with a surgical model under isoflurane general anaesthesia (Hepatology, 1996 ; 24 : 1452). The rats were maintained in normoglycaemia and normothermia. FHF rats were compared to control rats (CTL), to SHAM rats and to rats after partial hepatectomy (PH). Liver blood analyses were performed after 24 hrs. Intracranial pressure (ICP) was monitored, and brain water content was studied with a gravitometric method. Brain cortical samples were obtained after 12, 24, 36 hr and in coma stage 4, were immediately frozen in liquid nitrogen and stored at -80°C. AQP-4 mRNA expression was quantified by QRT-PCR, and AQP-4 protein content was measured by western blot on protein extracted from rat cortices. The western blot signal was obtained using cye-dye labelled secondary antibody and the membrane was then scanned using Typhoon° laser-scanner. The digitalized AQP-4 signal was normalized with an actin signal obtained in each lane.
**Results** : FHF rats developed acute cytolysis, cholestasis, liver failure, brain oedema and intracranial hypertension leading to death (mean survival : 44 hrs). Comparing to CTL (100%), we observed a decrease of AQP-4 gene expression : in PH, 50% of expression at 24 hrs and 40% at 36 hrs ; in FHF, 10% of expression at 24 hrs and 50% at 36 hrs. By western blot, the AQP4 protein level decreases to 60 to 70% of the control value in PH or FHF rats at 24 hrs or 36 hrs.
**Conclusions** : In this FHF model, there was no upregulation of the AQP-4 gene expression or of the AQP-4 protein level. At the contrary, AQP-4 content was significantly decreased compared to controls and equal to PH.