Metabolic profiling of human liver fibrosis

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Purpose/Introduction:
The liver may be affected by many pathological aggressions and then, liver dysfunction results in a variety of clinical signs and symptoms. However, some of these pathologies pose a diagnostic challenge as many different diseases show similar clinical signs. Moreover, liver biopsy assessment involves some degree of complication because of the proliferation of different grading and staging schemes. Liver fibrosis is characterized by the replacement of liver tissue by fibrous scar tissue as well as regenerative nodules, leading to progressive loss of liver function and to altered liver metabolism. Cirrhosis is the end-stage of this reaction and it represents a major change in the tissue. Global metabolic profiles may reflect the presence of a particular disease state. The aim of this study is to demonstrate the applicability of NMR spectroscopy biochemical profiling in human liver needle biopsy as support for chronic liver disease staging.

Subject and Methods:
Patients and tissue collection procedure Sixty eight samples of liver tissue were obtained from 68 chronic liver disease patients at Hospital Clinico Universitario of Valencia. The biopsy tissue was collected by anatomopathologist for routine histological analysis and informed consent was obtained for all patients. Liver tissue analyzed included 11 cirrhotic and 57 non cirrhotic, from 43 male and 25 female patients aged at 32-76. Histopathological grade and stage was assessed according to Batts-Ludwig classification (39). Sample weight ranged from 1 to 12 mg.
NMR spectroscopy of intact human liver tissue All spectra were recorded in a Bruker Avance DRX 500 spectrometer (Valencia, Spain) operating at a 1H frequency of 500.13 MHz and at 6C. Samples were spun at 4000 Hz. 2D experiments were acquired on selected samples for assignment purposes.
Spectral analysis All 68 spectra were processed using TopSpin 1.5 (Bruker Biopsin GmbH, Rheinstetten , Germany) and transferred to MATLAB (MathWorks Inc, 2006) using in-house scripts for data analysis. The chemical shift region including resonances between 2.00 and 4.40 ppm (the aliphatic region) was investigated. The spectra were normalized to the aliphatic spectral area between 2.0 and 4.4 ppm . The spectra were analyzed by Partial Least Squares Discriminant Analysis (PLS-DA) for detecting discriminating patterns and metabolites. Correlation with liver disease stage was explored by Pearson linear correlation analysis.

Results:
HR-MAS spectra from cirrhotic and non cirrhotic chrnonic liver disease tissue samples show great similarity because they are dominated by broad fatty acids signals. PLS-DA analysis provided partial separation between cirrhotic and non cirrhotic samples (Figure 1). The Major metabolic differences correspond to the levels of some unsaturated fatty acids, choline, phosphocholine, glucose, glutamate, glutamine, aspartate and phosphoethanolamine. Spectral signal integration was performed over the signals of these metabolites. Further analysis of the levels of glutamate and glutamine also showed some correlation with the disease stage (Glutamate, Pearson correlation coefficient of 0.39 and p-value 0.04; Glutamine, Pearson correlation coefficient of -0.51 and p-value 0.03). The mean values for these metabolites in the different disease stages have been represented in Figure 2.
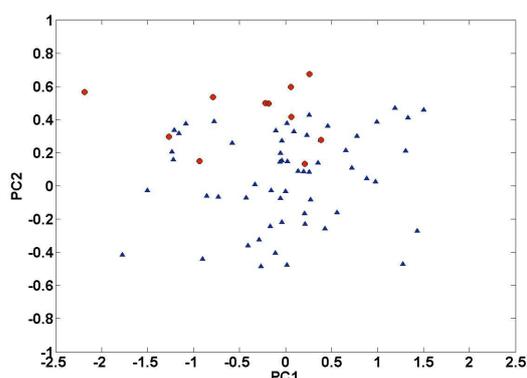

Figure 1 PLS-DA scores plot (R2 = 30.2%, Q2 = 78.2%) for discrimination between cirrhotic (red circles) and non cirrhotic (blue triangles) chronic liver disease biopsies.
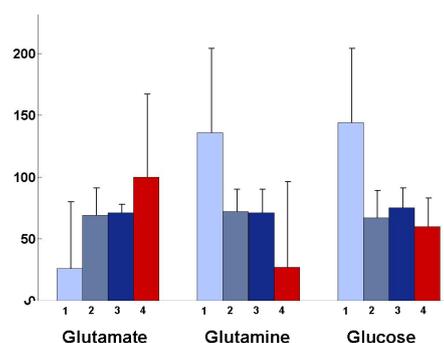
Figure 2. Bar graphs for metabolites involved in ammonia detoxification by the liver at the different liver disease stages.

Discussion/Conclusion:
Metabolic profiles of chronic liver disease biopsies provided differential patterns between cirrhosis and non cirrhosis and allow the determination of progressive metabolic alterations associated to chronic hepatic disease. In this work, we report that metabolic alterations associated to liver disease stage affect essential metabolic processes beyond lipid metabolism. Early stages of chronic liver disease seem to have important metabolic consequences including increased glutamate and decreased glutamine and glucose. Overall, this work suggests that the additional information obtained by NMR metabolomics applied to needle biopsies of human liver may be useful for assessing metabolic alterations and liver dysfunction in chronic liver disease.

Acknowledgements