Metabolic phenotyping of cirrhotic liver samples by desorption electrospray ionization mass spectrometry imaging (DESI-MSI)

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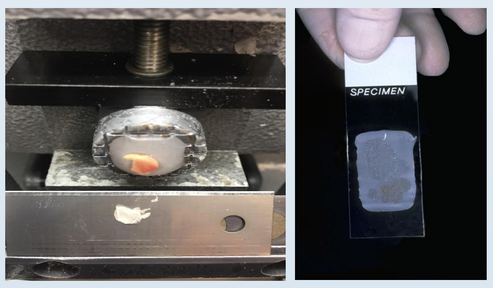
Introduction

- Cirrhosis represents the final outcome of several pathological conditions. Since different aetiopathogenesis may show similar histologic features, the histopathologist may struggle to detect the primary liver disease without a complete clinical history.
- Some patients within the spectrum of autoimmune liver diseases present with characteristics of both **autoimmune hepatitis (AIH)** and **cholestatic liver disease** (*i.e.* **primary biliary cirrhosis (PBC)**). These two conditions may be difficult to classify and since patients within each diagnosis may present with a range of clinical, serological, biochemical and histological findings, the differential diagnosis between them may be a challenge.
- Identically, **non-alcoholic steatohepatitis (NASH)** and **alcoholic liver disease (ALD)** have similar pathogenesis and histopathology. Correct diagnosis of these two conditions is crucial as it has important therapeutic and prognostic implications for patients.
- Since DESI-MS allows us to correlate MSI data with histological feature, topographically localised biochemical information can be obtained and used to supplement conventional histological classification systems. Therefore, DESI-MSI was used to understand the metabolic hallmarks of different liver diseases and use this information to augment diagnostics.

Methods

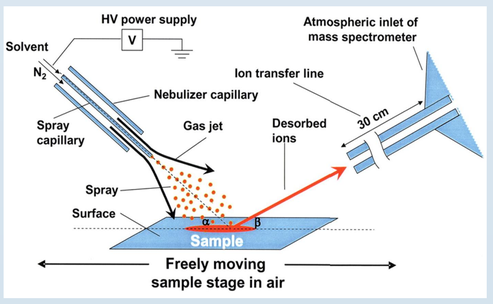
Fresh frozen tissue samples

- Samples stored at -80°C prior cryosectioning



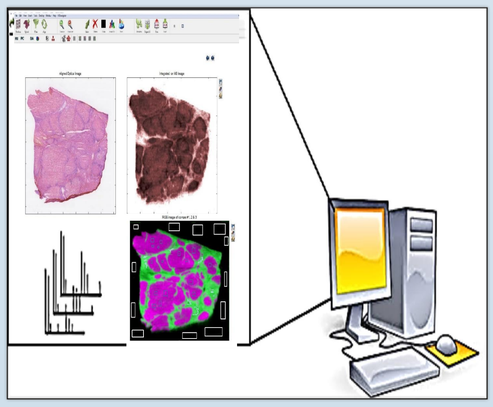
Cryosectioning

- Sections cut at 10µm at -18°C and stored at -80°C prior DESI-MSI



DESI-MSI

- Mass to charge (*m/z*) range – 150 – 1500
- Solvent 95:5 methanol / water
- Flow rate 1.5 µL/min
- Mass resolution 100,000



Data analysis

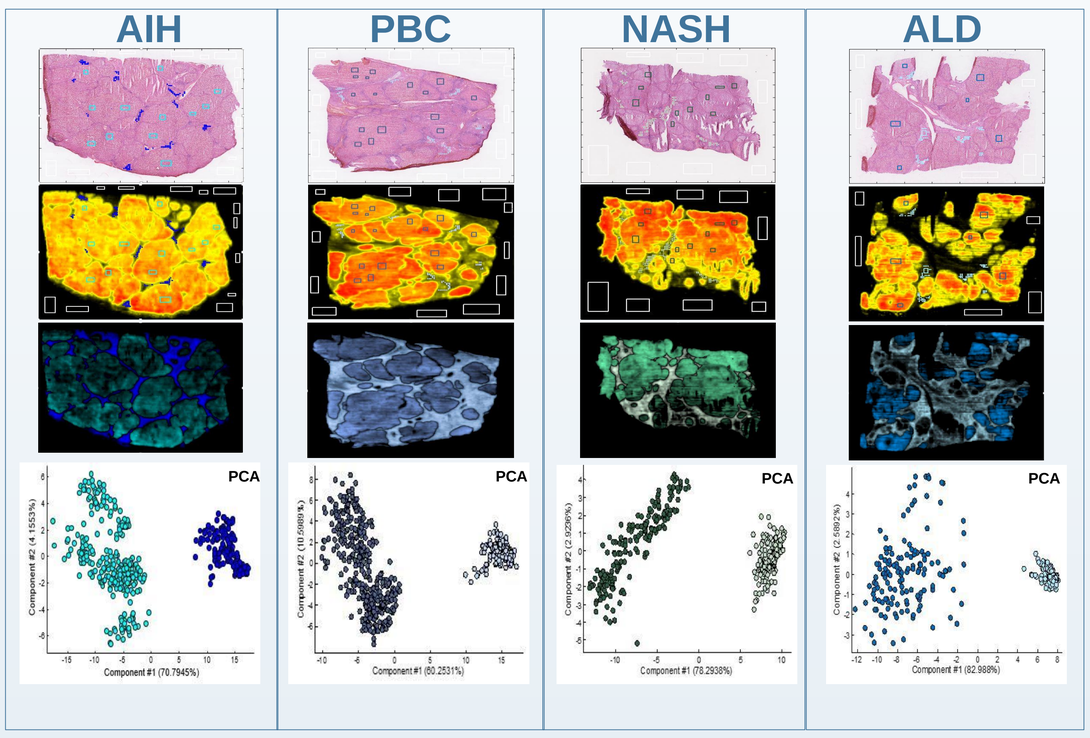
- Optimized pre-processing workflow
- Image co-registration for accurate co-localization of mass spectrometry and histological features
- Supervised maximum margin criterion for enhanced tissue specific marker recovery
- In house mass spec imaging toolbox

References

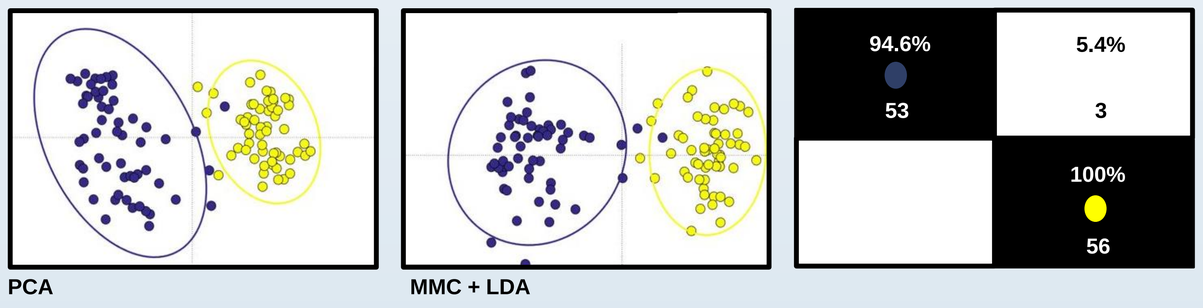
1. Boberg, K.M., *et al.* Overlap syndromes: The International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. *Journal of Hepatology*, 54(2), 374-385
2. Scaglioni, F., *et al.* ASH and NASH. *Digestive diseases*, 29(2), 202-210
3. Takats, Z., et al., Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science*, 2004. 306(5695): p. 471-473

Results

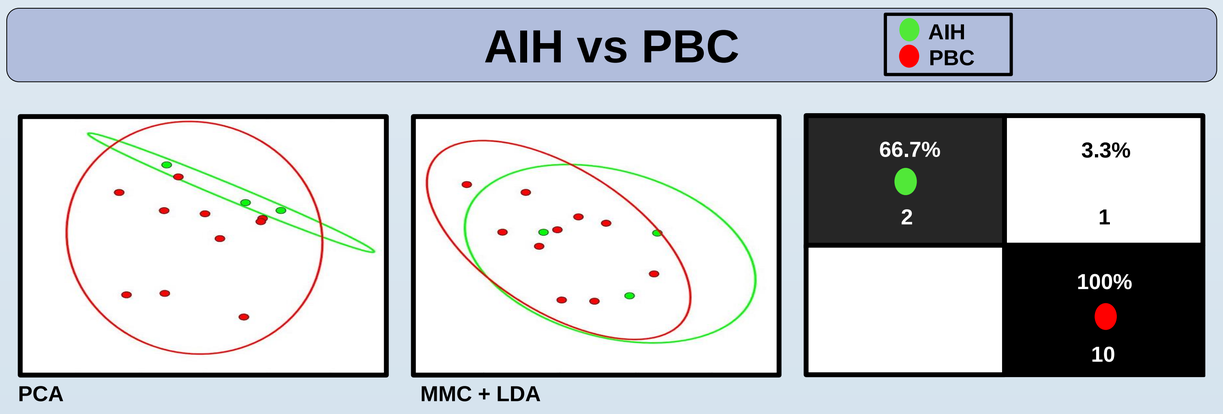
Each individual sample was subjected to statistical analysis. All pixels of the samples were classified in the different tissue types based on the corresponding histological image and performing supervised analysis using PCA (principal component analysis) and recursive maximum margin criteria (RMMC/LDA).



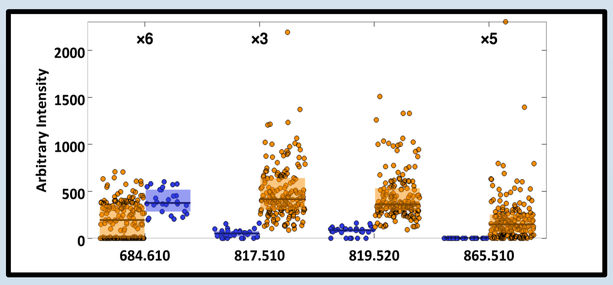
Fibrosis vs nodules



AIH vs PBC



AIH and PBC differentiation



AIH ↑ 684.610 – Cer(d42:1)

PBC ↑ 817.510 – PG(40:8)
819.520 – PG(40:7)
865.510 – PG(44:6)

Conclusion

- In each sample, nodules and fibrotic tissue reveal different characteristics information (lipid profile) directly correlated with histological information.
- Lipid distribution can differentiate the nodules from fibrotic tissue in each cirrhotic liver disease tested.
- DESI-MSI is an useful technique which can significantly contribute to diagnostic of cirrhotic liver diseases. Tissue samples representing AIH and PBC can be separated when using this technique.