Signature of circulating hepatic proteins detects fibrosing-steatohepatitis in progressive Non-alcoholic Fatty Liver Disease

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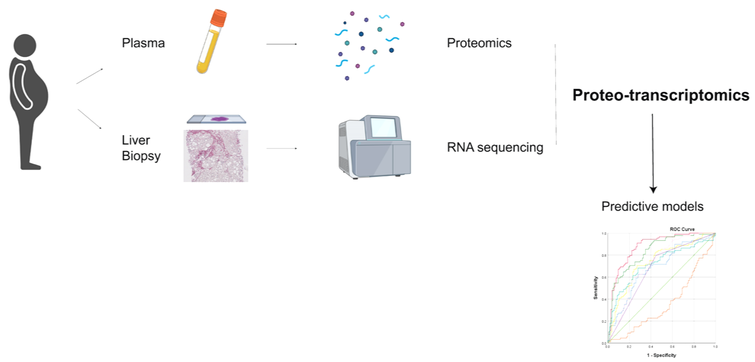
NAFLD: Diagnostics and non-invasive assessment

Proteo-transcriptomics in NAFLD



1 Introduction & Aims

Non-alcoholic fatty liver disease (NAFLD) is considered as the hepatic manifestation of the metabolic syndrome and changes in circulating blood proteins have been associated with advanced NAFLD. Yet, it is still unclear which proteins originate from the liver and how these alter during disease progression.

2 Method

The cohort comprised 256 patients from the European NAFLD Registry with histologically proven NAFLD identified at four specialist centres. The histological semi-quantitative NASH CRN system was used to score the biopsies. 191 plasma samples were processed for proteomics analysis using the SomaScan™ platform. In a subset of 51 cases, snap-frozen liver biopsies underwent high-throughput RNA sequencing (RNAseq). Integrative analysis of these data with publicly available single-cell RNAseq data was used to identify cell of origin. Binary logistic modelling was implemented to predict disease activity.

3 Results

Comparing patients with advanced fibrosis (F3-4) to mild disease (F0-2) in the discovery cohort of 191 plasma samples identified 156 differentially expressed proteins, while stratifying based on a NAFLD Activity Score (NAS) ≥4 identified 79 proteins. Of these, 34 proteins were common to both analyses, including AKR1B10, APOF, THBS2 and TREM2 (Figure 1).

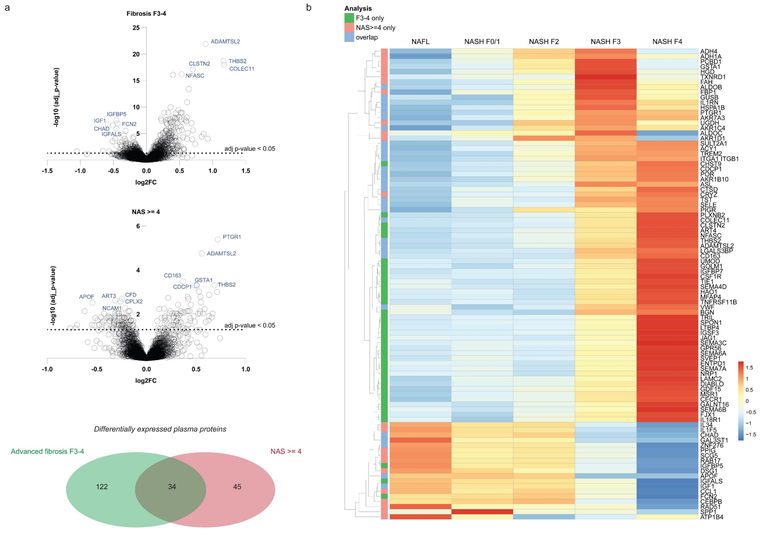


Figure 1. Proteomics analysis of 191 plasma samples from patients with histological proven NAFLD. *(a) Differentially expressed proteins when stratifying patients based on fibrosis stage F3-4 versus baseline F0-2 and based on a high disease activity score NAS≥4. Venn diagram shows the overlap between the two different analyses. (b) Heatmap showing expression of top 35 most significant proteins associated with fibrosis and disease activity.*

3 Results - Continued

To determinate which proteins originate from the liver, we performed a correlation analysis between plasma proteins identified by the F3-4/NAS≥4 analyses and mRNA within 51 patients, finding 32 proteins/mRNAs reaching the significance threshold. Deconvolution by single-cell RNAseq data indicated the different hepatic cellular changes during disease progression, such as AKR1B10, APOF and GDF15 to originate from epithelial cells, ADAMTSL2 and THBS2 from fibroblasts and TREM2 from macrophages (Figure 2a). Finally, to assess potential utility within a non-invasive diagnostic pathway to detect fibrosing-steatohepatitis defined as the presence of NASH, NAS≥4 and F≥2, we performed logistic regression analysis. Backward elimination of variables identified a composite model that could predict NASH+NAS≥4+F≥2 with an Area Under the Curve of 0.878 based on markers including circulating ADAMTSL2, AKR1B10, CFHR4 and TREM2 (Figure 2b).

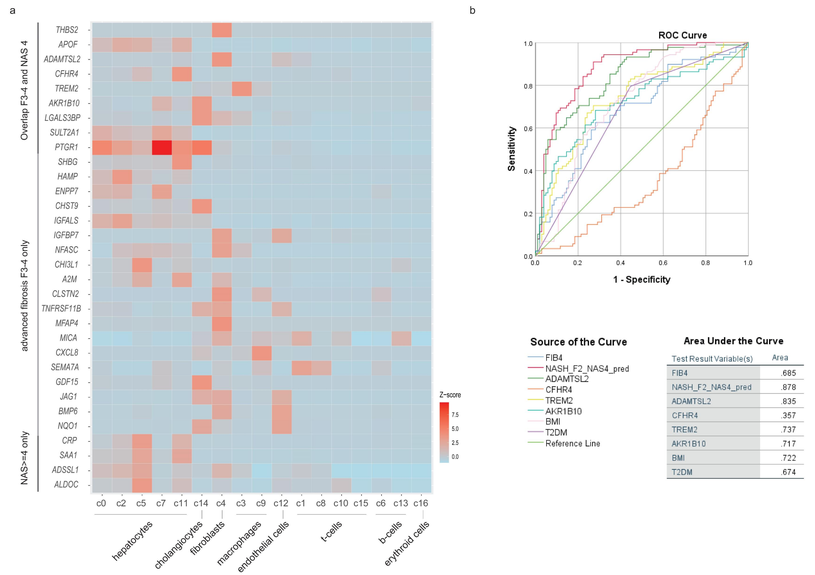


Figure 2. Proteo-transcriptomics correlation. *(a) Integrated single cell RNA sequencing analysis to deconvolute the 32 signature of proteins associated with hepatic mRNA. (b) Predictive modelling to identify NASH+NAS≥4+F≥2 using plasma from patients with histological characterised NAFLD (n=191) compared with the FIB4 score.*

4 Conclusions

We showed that circulating proteomic changes reflect grade of steatohepatitis and stage of fibrosis that may be used to assess disease severity.

5 Acknowledgements

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