# Early prediction of progressive fibrogenesis in NAFLD-NASH patients using blood-based biomarkers

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## Introduction

The incidence of NAFLD-related fibrosis is rapidly increasing worldwide. Fibrosis is the main determinant for mortality and is therefore an important clinical readout. Detection of fibrosis via a liver biopsy is still the golden standard but this is an invasive procedure with risk for complications, sampling and reading errors. Fibrosis is a dynamic process and currently no validated diagnostic/prognostic methods exist, especially biomarkers which can detect the early onset and progression of fibrosis.

**Aim:** Identification of a mechanism-based biomarker panel which is prognostic for NAFLD-related fibrosis and enables early identification of people at risk with active fibrogenesis.

## Method

- Bio-banked Formalin-Fixed Paraffin Embedded (FFPE) human liver tissues were used to identify key pathways involved in NASH and fibrosis.
- Preclinical time-course studies were performed to determine molecular signature for active fibrosis using deuterated water labeling and dynamic -omics technologies. These enabled us to identify a set of genes related to fibrosis at a moment where no pathological fibrosis was observed yet.
- This molecular signature was translated into a set of candidate blood based biomarkers coupled to matrix deposition and fibrosis progression.
- This set was evaluated in longitudinal samples from the HELIUS and ANCHOR cohorts (n=80) at baseline and after 6 year follow up. A set of 12 biomarkers related to progressive fibrosis was analyzed in baseline samples of individuals which developed fibrosis (n=40) or did not develop fibrosis (n=40) as determined by FibroScan and ELF test at the 6 year follow-up measurement.

## RNAseq of FFPE sections from Human liver biopsies

| Number of DEGs (Pval<0.01) | | F1 | F2 | F3 | F4 |
|---|---|---|---|---|---|
| | | F0 | F0 | F0 | F0 |
| F1 | F0 | 629 | 200 | 140 | 168 |
| F2 | F0 | | 1049 | 594 | 599 |
| F3 | F0 | | | 5016 | 3124 |
| F4 | F0 | | | | 6299 |

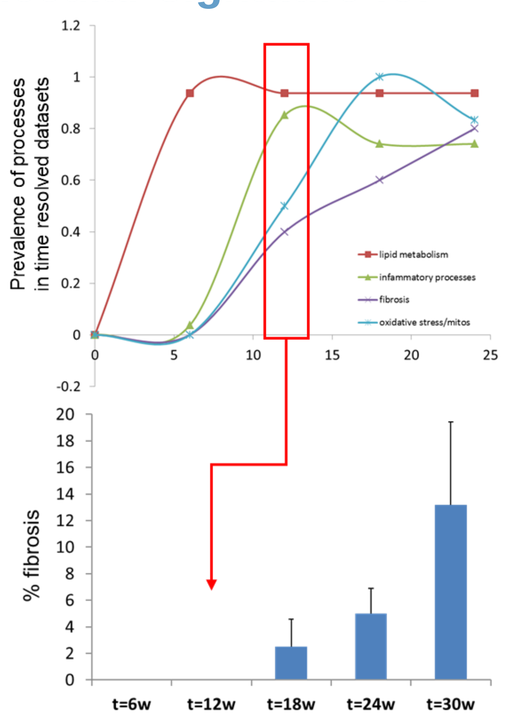
**Table 1:** Number of differentially expressed genes per F-score as compared to NASH without fibrosis (F0)

Transcriptome analysis shows over 6000 differentially expressed genes in severe fibrosis (F4) samples as compared to NASH without fibrosis.

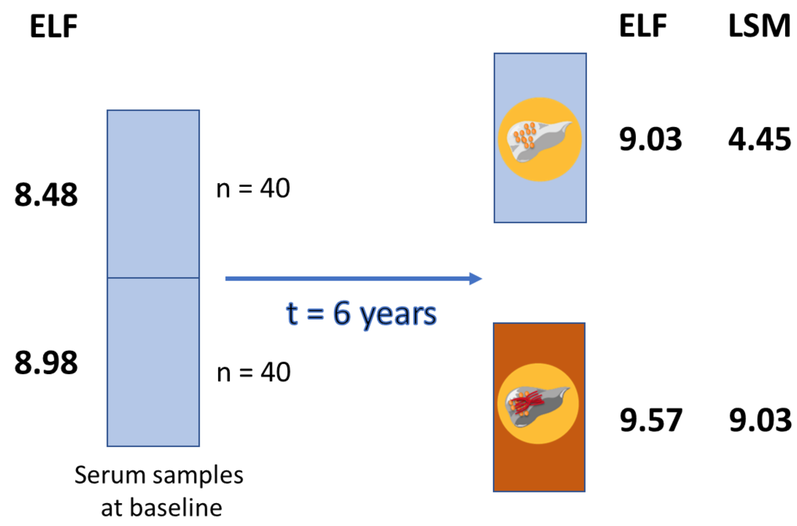
## Mouse study to identify molecular signature for active fibrogenesis

Next step was to apply deuterated water technology to label all newly formed collagens in liver from HFD-fed mice in a time-dependent manner.

We identified a molecular signature of 232 genes closely correlating to collagen synthesis (PMID 29276754). Part of the signature was present before pathology was detectable

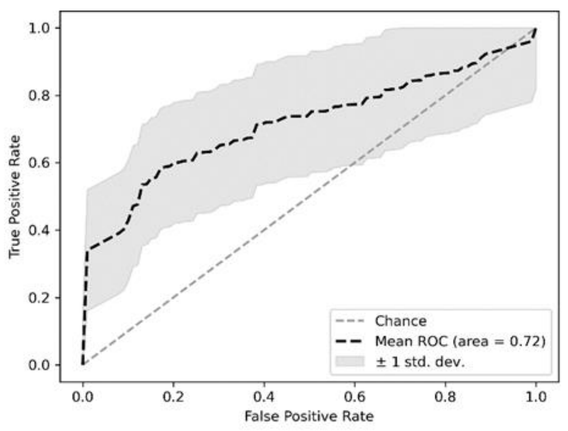



## Analysis of biomarker set in individuals with 6 years follow-up (1)




In total, 12 biomarkers related to progressive fibrosis and which could be detected in human serum were analyzed at baseline and after 6 years follow-up. 80 individuals were analyzed of whom 40 showed development of fibrosis after 6 years

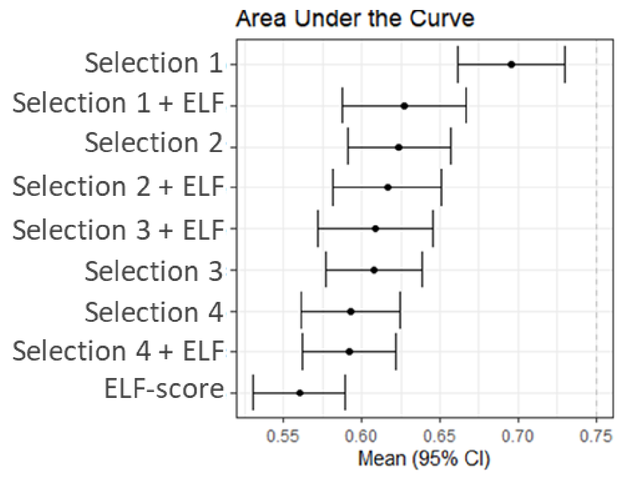
## Analysis of biomarker set in individuals with 6 years follow-up (2)




A set of two biomarkers was identified which at baseline predicted fibrosis development at 6 years follow up. (AUROC 0.70; sensitivity of 0.72 and specificity of 0.62)

## Comparison with ELF

Using baseline serum data, Random Forest analysis showed average predictive value for (no fibrosis, *moderate fibrosis and severe fibrosis) at 6 years follow up by ELF score of 62% (AUROC=0.62; Kappa 0.02).




Combination of ELF with our biomarkers improved the prediction substantially with optimal predictive value of 68% (AUROC=0.68; Kappa = 0.25;

## Conclusions

- By integration of mechanistic mouse studies and human clinical samples we identified a set of circulating proteins related to the active fibrogenesis process
- A selection of this panel was used to predict fibrosis at a 6-year interval, enabling early identification of individuals at risk with active fibrogenesis

**Future plans:**

- Further validation of the prognostic biomarker set in larger patient cohorts with follow-up data over time
- Improving the predictive value of the biomarker set by adding clinical data