Prognostic value of the TLM3 biomarker panel for early detection of fibrosis development in MASLD within the general population

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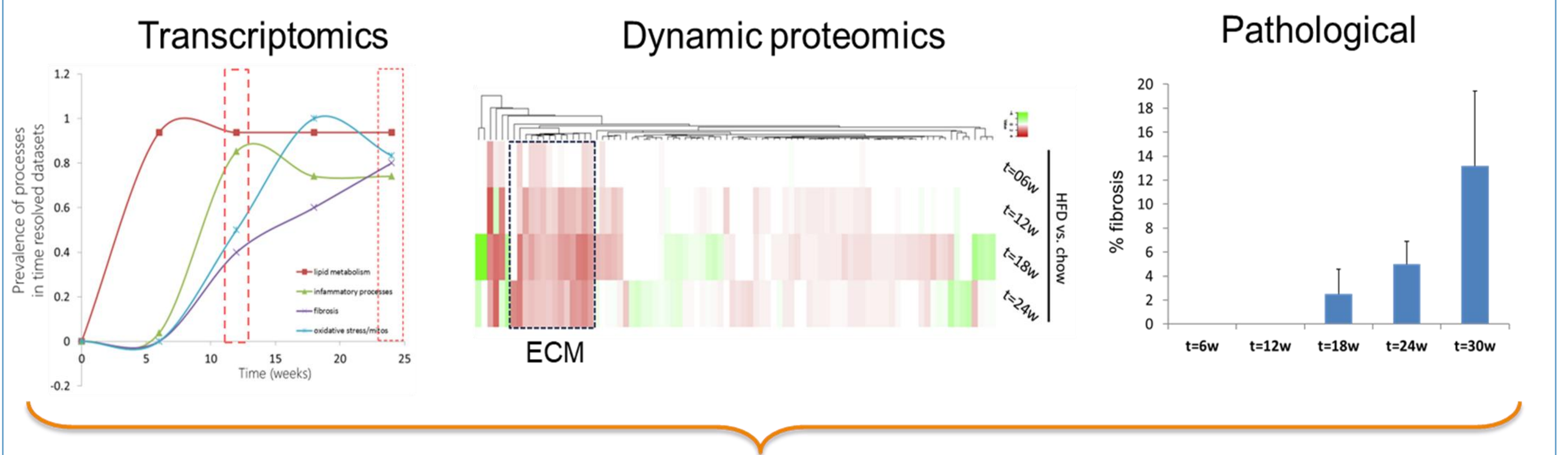
1. Background and aims

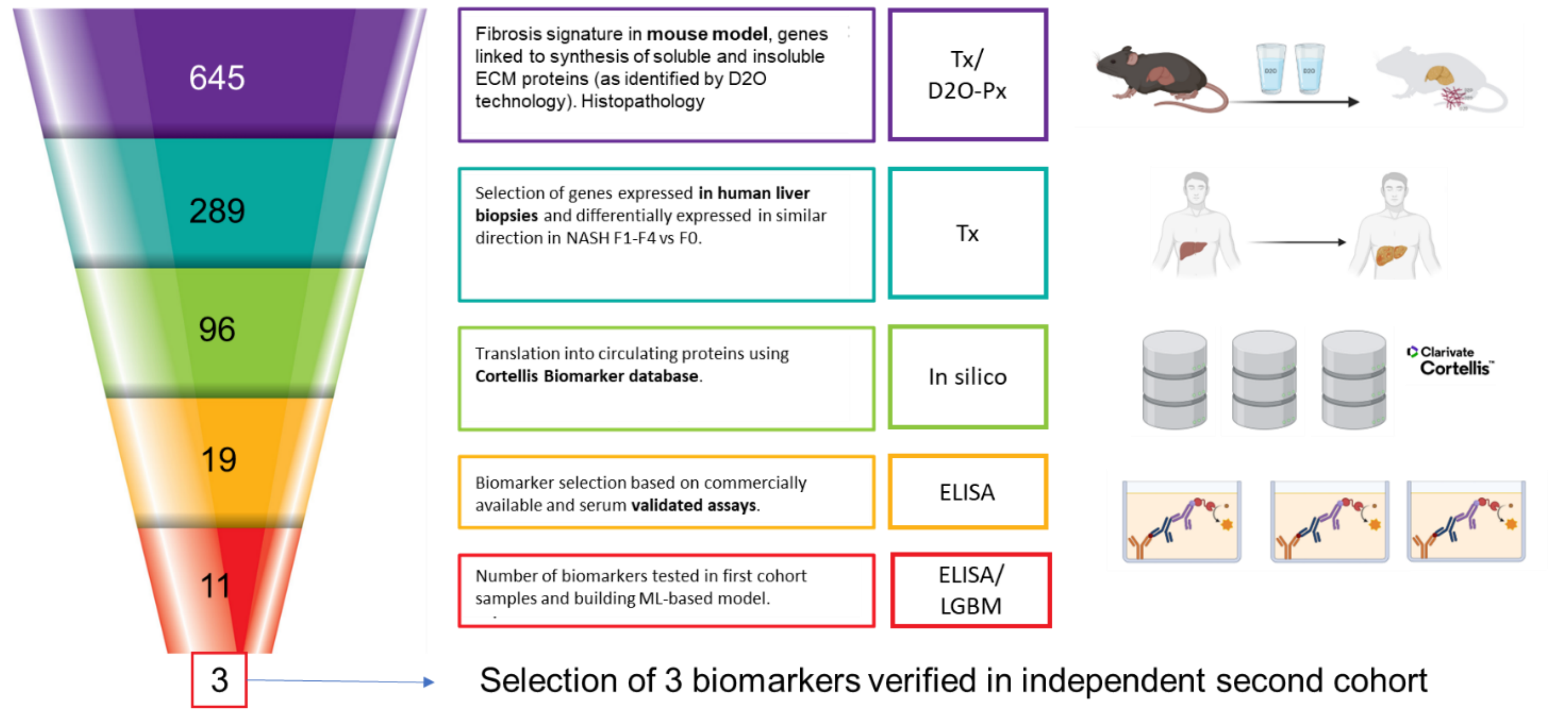
Fibrotic MASLD is associated with increased morbidity and mortality, often remaining asymptomatic until advanced stages of disease. Predicting fibrosis onset and progression would improve risk stratification and treatment allocation. This study aims to investigate whether a previously identified fibrosis biomarker panel for active fibrogenesis (TLM3) can serve as a prognostic marker panel for fibrosis development in a population at cardiometabolic risk of fibrotic MASLD.

2. Biomarker identification approach

A fibrosis signature was identified using a translational diet-induced MASLD mouse model (LDLr-/-.Leiden), focused on the mechanism of collagen protein synthesis using hepatic gene expression (NGS) in combination with new extracellular matrix deposition detected by dynamic D_2O-labeling. 645 DEGs correlated with new extracellular matrix protein formation.



To translate these findings to humans, gene expression profiles were analyzed in liver biopsies and serum biomarkers from patients with histologically characterized MASLD and variable degrees of fibrosis



Fibrosis signature in **mouse model**, genes linked to synthesis of soluble and insoluble ECM proteins (as identified by D2O technology). Histopathology

Selection of genes expressed **in human liver biopsies** and differentially expressed in similar direction in NASH F1-F4 vs F0.

Translation into circulating proteins using **Cortellis Biomarker database**.

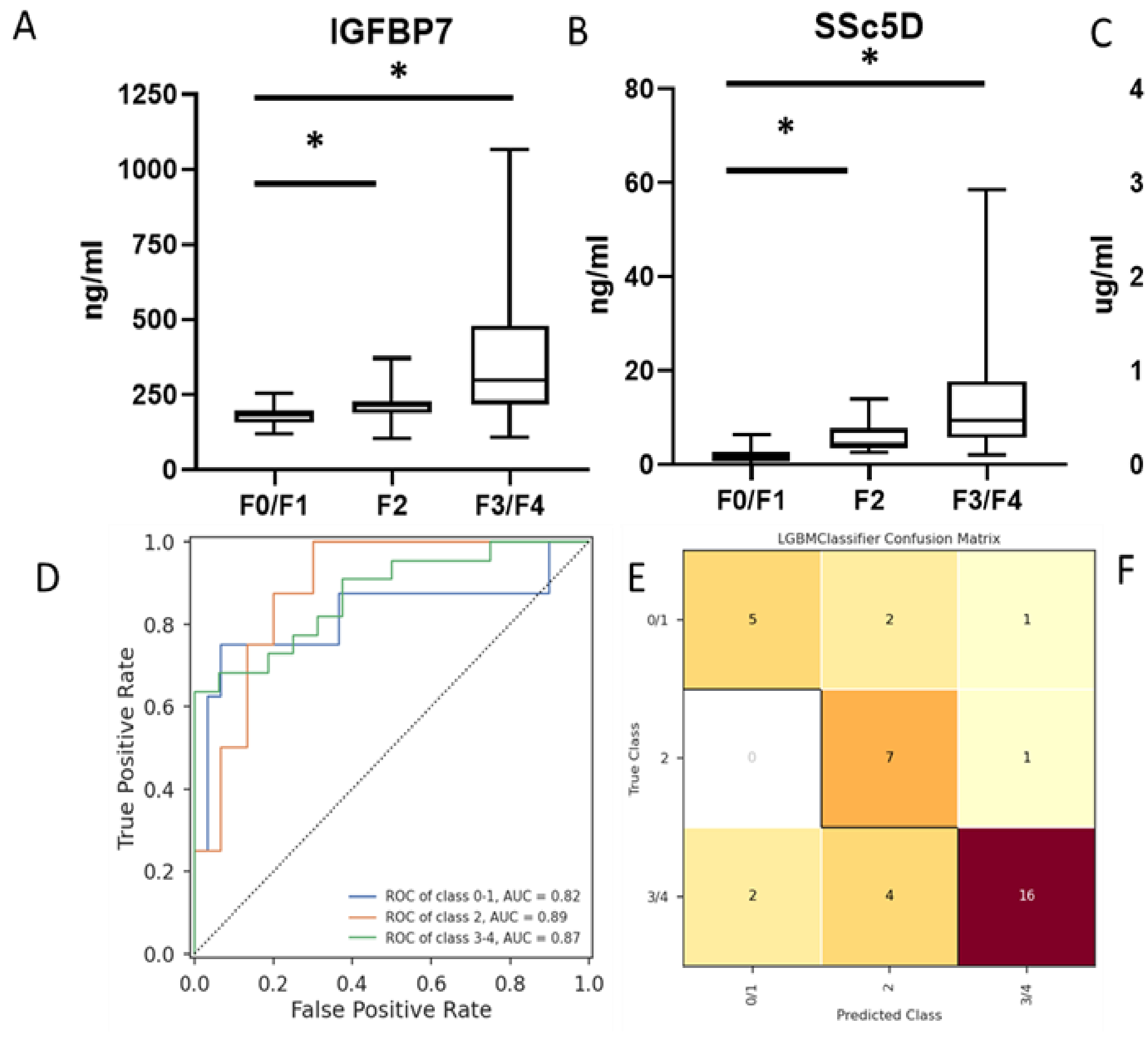
Biomarker selection based on commercially available and serum **validated assays**.

Number of biomarkers tested in first cohort samples and building ML-based model.

Selection of 3 biomarkers verified in independent second cohort

4. TLM3 shows great performance in validation cohort

A machine learning model was built using the 11 selected candidate biomarkers, leading to a model (TLM3): IGFBP7, SSc5D and SEMA4D.



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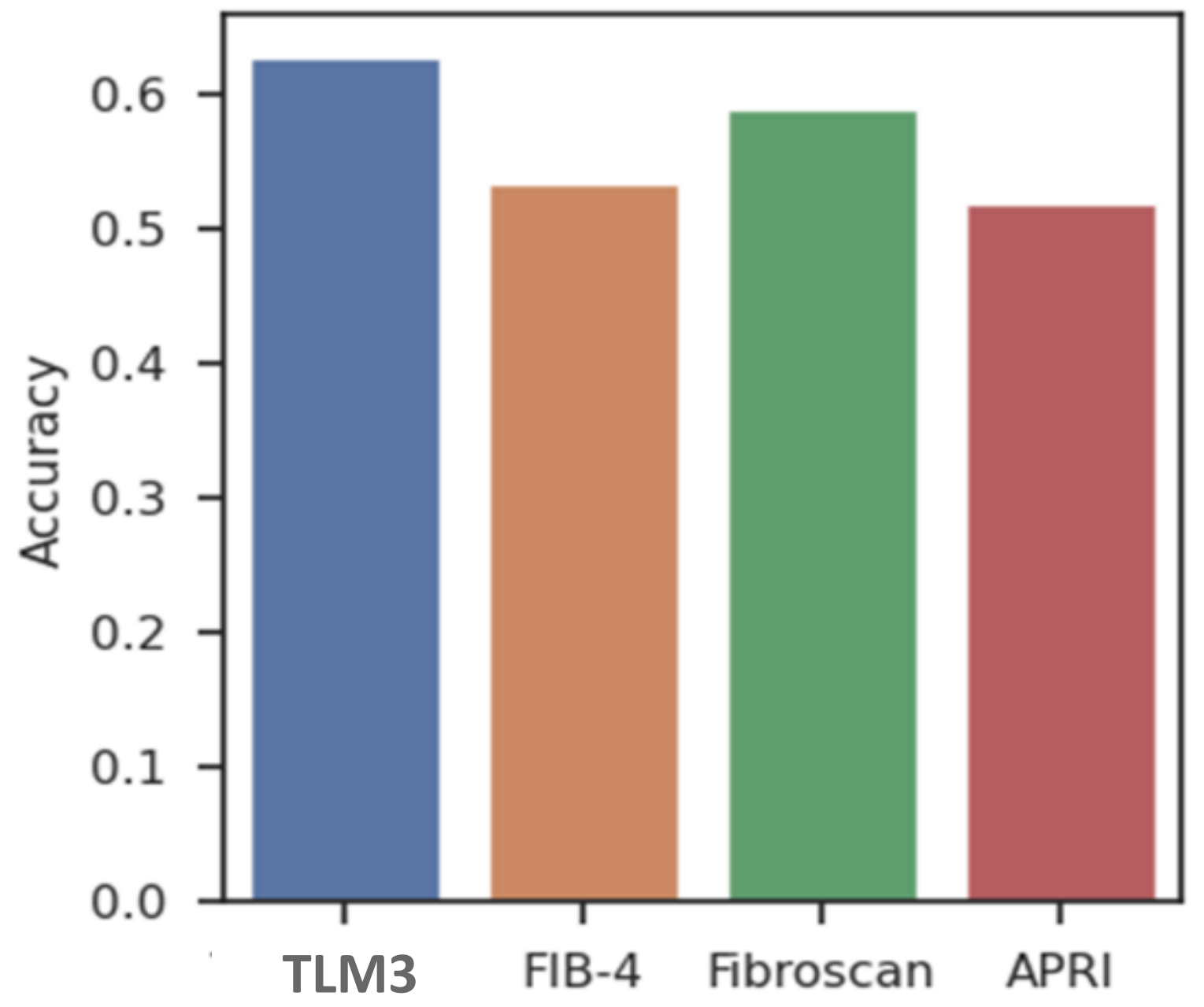
	Sensitivity	Specificity
F0-F1	0.625	0.903
F2	0.875	0.960
F3-F4	0.727	0.700

In a validation cohort (n=128 patients) the TLM3 model achieved an area under the receiver operating curve (AUROC) of 0.82 for fibrosis stage F0/F1, a robust AUROC of 0.89 for fibrosis stage F2, and a strong AUROC of 0.87 for distinguishing fibrosis stages F3 and F4 (Figure D).

5. Overall accuracy of the model outperformed Fibroscan, FIB-4 and APRI

The TLM3 model was compared with FIB-4 (sensitivity, specificity, precision) and FIB-4, Fibroscan and APRI (overall accuracy).



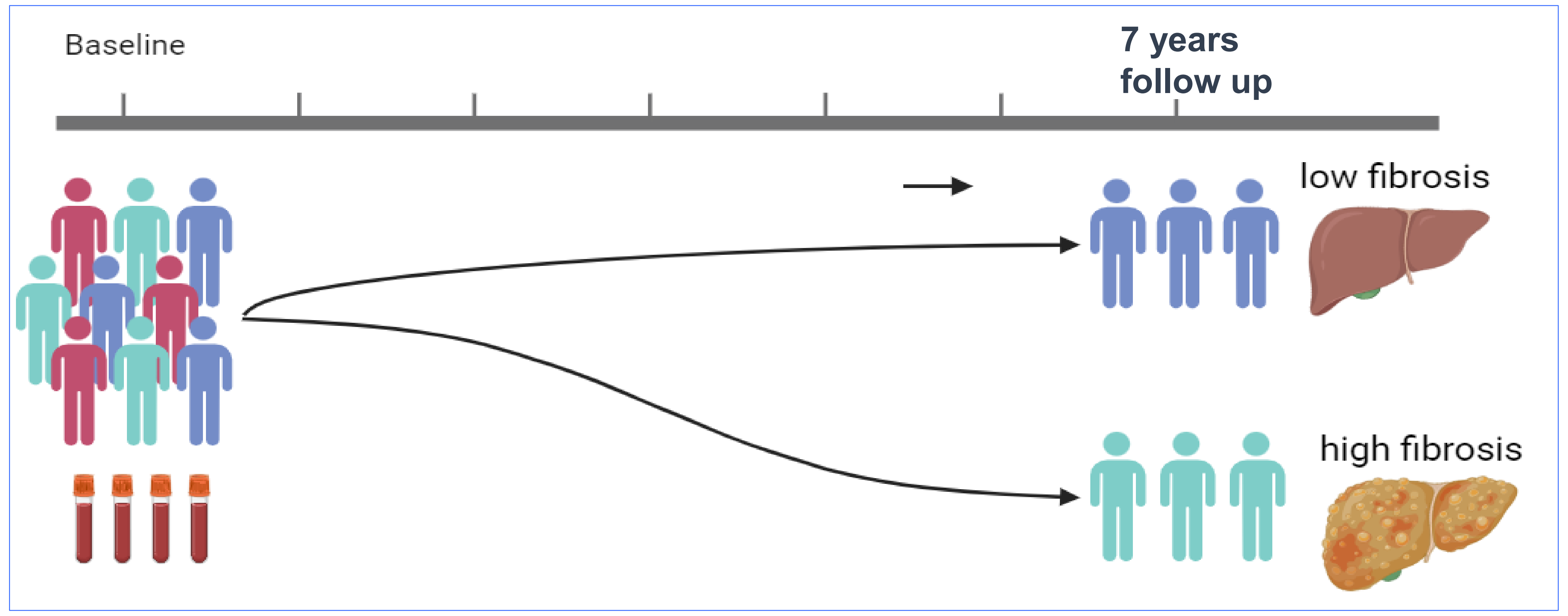
Perfomance metrics FIB-4	Sensitivity	Specificity	Precision
F0/1	0,70	0,83	0,52
F2	0,43	0,75	0,28
F3/4	0,28	0,64	0,74

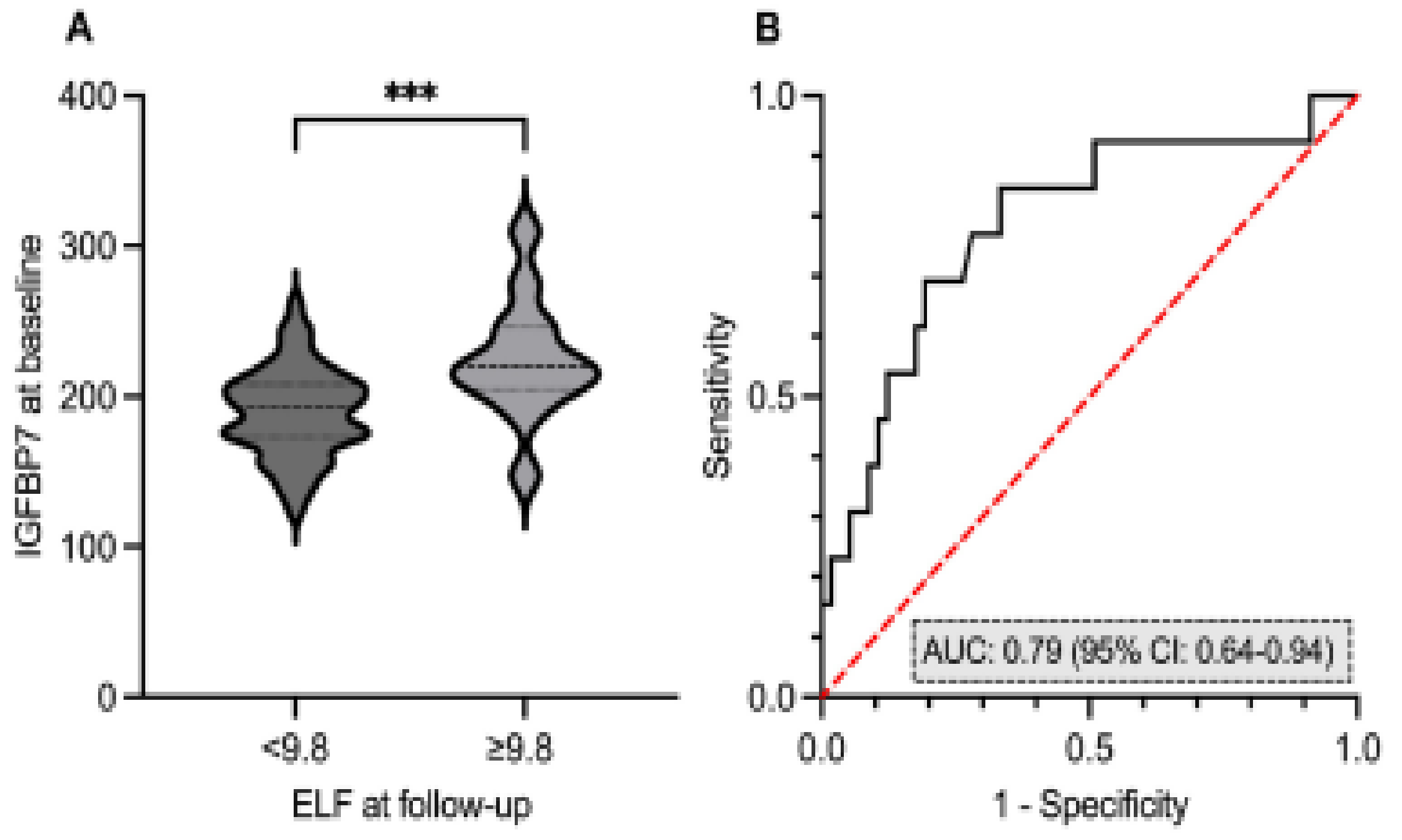
Perfomance metrics TLM3	Sensitivity	Specificity	Precision
F0/1	0,69	0,82	0,76
F2	0,50	0,87	0,36
F3/4	0,68	0,78	0,76

The TLM3 was similar to FIB-4 for F0/F1 and outperformed FIB-4 for F2 and F3-F4. TLM3 also had better accuracy than the other NITs that were tested.

6. TLM3 is predictive 7 year before diagnosis of fibrosis

TLM3 was measured in baseline serum from individuals at risk of MASLD from the general population HELIUS-cohort and correlated to established fibrosis proxies (ELF, VCTE and FIB4) at 7 years follow-up.





Serum levels of IGFBP7, Ssc5D, Sema4D, VCAN, THBS1 and TNC at baseline correlated with fibrosis proxies at follow-up. IGFBP7 at baseline was able to predict new onset fibrosis, defined as ELF >9.8 at follow-up in participants with ELF <9.8 at baseline, with an area under the curve (AUC) of 0.79 (95% CI: 0.64-0.94).

7. Conclusion

Using the dynamics of disease mechanism in a translational preclinical model we were able to identify and validate the TLM3 biomarker set which was able to accurately diagnose fibrosis stage in MASLD patients.
Moreover, due to the involvement in early disease processes, the biomarker set was able to predict fibrosis outcome in MASLD 7 years ahead of diagnosis.

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