The urine steroid metabolome combined with machine learning performs similarly to established non-invasive markers to identify advanced MASLD fibrosis.

EASL CONGRESS
7-10 May 2025
Amsterdam, the Netherlands

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Introduction

Metabolic dysfunction-associated steatotic liver disease **(**MASLD) affects around 30% of people worldwide. The strongest predictor of clinical outcome in patients with MASLD is liver fibrosis stage. Due to limitations with the liver biopsy, there is a need to develop reliable non-invasive tests to identify patients with advanced fibrosis. There are currently no validated non-invasive urine tests to stage MASLD; The TrUSt-NAFLD study aimed to prospectively validate the use of urine steroid metabolite analysis coupled with machine learning to diagnose and stage MASLD.

Method

Between May 2021 and November 2023, 461 patients were recruited to the TrUSt-NAFLD study with ethical approval. 151 healthy control patients were recruited from the Oxford Biobank and 310 patients with biopsy-proven MASLD were recruited from 8 centres across the United Kingdom. Urine samples were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS) for 19 steroid metabolites. A machine learning based classifier, Generalised Matrix Learning Vector Quantisation (GMLVQ), was used to classify patients into early (F0-2) or advanced (F3-4) fibrosis.

Results

The TrUSt-NAFLD cohort is a representative cohort of patients with MASLD who underwent a clinically indicated liver biopsy to determine MASLD stage and a matched healthy control cohort. The urine steroid metabolite analysis coupled with GMLVQ performed similarly to other established non-invasive biomarkers (AUROC = 0.65), including FIB-4 (0.72), NFS (0.69) and VCTE (0.69) at distinguishing early from advanced fibrosis. The early fibrosis cohort had significantly higher concentrations (microgram steroid per g creatinine) of androsterone (162.8 vs 115.9; p<0.01), etiocholanolone (353.7 vs 199.8; p<0.01) and THA (113.0 vs 90.2, p=0.04). It was also able to distinguish healthy controls from patients with MASLD (AUROC = 0.79).

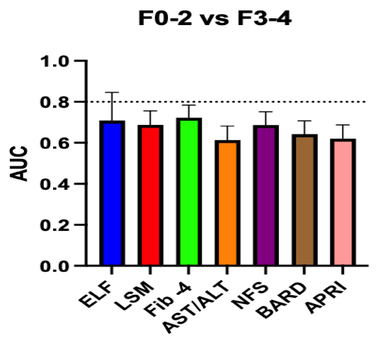
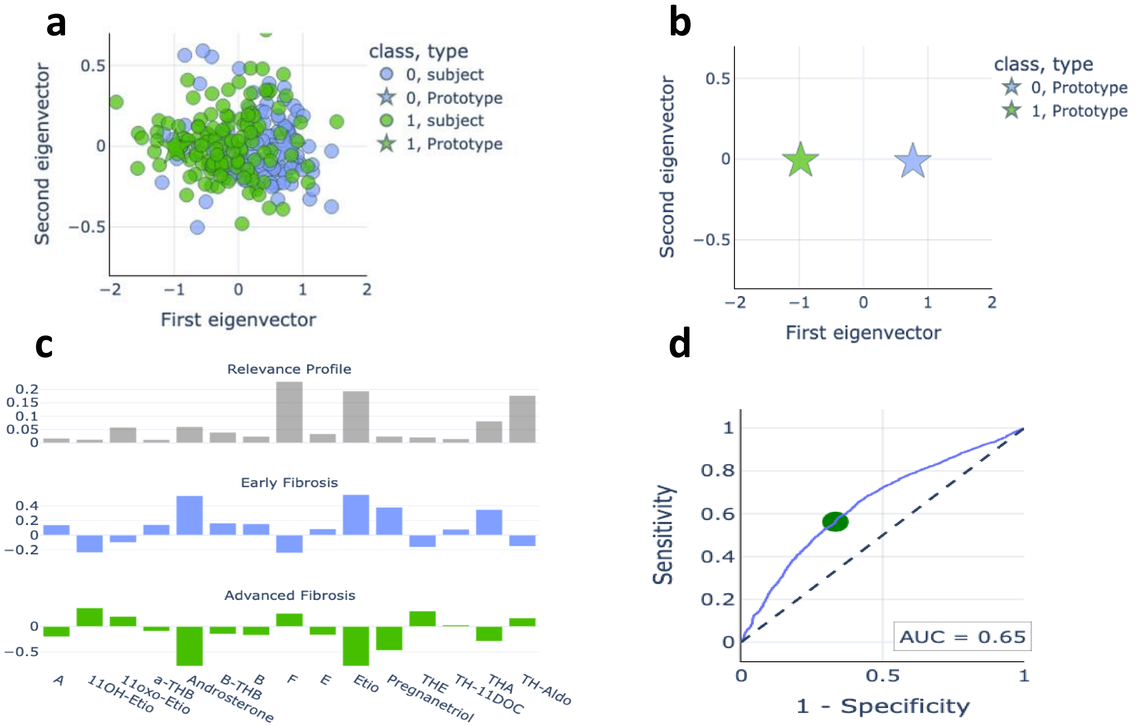


Figure 1: Chart comparing performance of commonly used non-invasive test scores in discriminating between early (F0-2) and advanced (F3-4) fibrosis

	Early Fibrosis (F0-2)	Advanced Fibrosis (F3-4)	p value
	(N=122)	(N=165)	
Age	38.9 **48.0** 58.1	50.7 **59.0** 64.0	p<0.01[2]
Sex: (% male)	72/122 (59.0%)	83/165 (50.3%)	p=0.14[1]
BMI (kg/m^2)	30.4 **35.6** 40.6	30.1 **34.4** 39.4	p=0.30[2]
Diabetes	47/121 (38.8%)	114/165 (69.1%)	p<0.01[1]
Alcohol Use	51/121 (41.8%)	62/165 (37.6%)	p=0.43[1]
Haemoglobin (g/dL)	134.9 **150.0** 158.0	132.0 **143.0** 153.8	p=0.01[2]
Platelet Count (x10^9/L)	198.7 **255.0** 297.7	184.2 **227.0** 267.0	p<0.01[2]
ALT (iu/L)	29.0 **43.0** 72.0	29.0 **47.5** 64.0	p=0.88[2]
AST (iu/L)	26.0 **36.0** 50.1	30.0 **41.0** 58.8	p=0.03[2]
Bilirubin (µmol/L)	8.0 **11.0** 14.0	8.0 **11.0** 15.0	p=0.94[2]
Albumin (g/L)	40.0 **43.0** 45.0	39.0 **42.0** 44.5	p=0.07[2]
ALP (iu/L)	70.2 **84.0** 108.0	67.0 **91.0** 113.3	p=0.88[2]
HbA1C (mmol/mol)	35.9 **41.5** 52.2	40.0 **49.0** 62.0	p<0.01[2]

Table 1: Characteristics of patients with early fibrosis and patients with advanced fibrosis. BMI: Body Mass Index. ALT: alanine transaminase, AST: aspartate transferase, ALP: alkaline phosphatase, HbA1c: glycated haemoglobin Data are expressed as (Q1, **median,** Q3) (Significant p values have been boldfaced. N is the number of non-missing value. [1]Pearson. [2]Wilcoxon.



Figures 2a – 2d. GMLVQ analysis of urinary steroid profiles from early fibrosis (F0-2) vs. advanced fibrosis (F3-4). a: each circle represents a participant, blue for early fibrosis and green for advanced fibrosis. b: The stars represent the prototype for early fibrosis (blue) and advanced fibrosis (green). A prototype is a particular pattern of steroid metabolites generated by GMLVQ based on training data. c: The relevance profiles and prototypes are displayed for each group. The relevance profile details how much each metabolite is contributing to the prototype. In this analysis, F (cortisol) is the most important shown by the highest grey bar. It is displayed below the axis in the early fibrosis group (blue) and below the axis in the advanced fibrosis group (green). This demonstrates that for cortisol, if an unknown sample has a lower amount on cortisol, it is more likely to be from an early fibrosis patient. d: the performance of GMLVQ in discriminating between early fibrosis and advanced fibrosis patients is displayed with an AUC. A: 11-dehydrocorticosterone, 11OH Etio: 11-OH-Etiocholanolone, 11oxo Etio: 11-oxo-Etiocholanolone, α THB: α-tetrahydrocorticosterone, β THB: β tetrahydrocorticosterone, B: corticosterone, F: Cortisol, E: Cortisone, THE: Tetrahydrocortisone, TH-11-DOC: tetrahydro-11-deoxycortisol, THA: Tetrahydro-11-dehydrocorticosterone, TH-Aldo: tetrahydroaldosterone.

Conclusions

The urine steroid metabolome provides a non-invasive method of assessing liver phenotype based on non-classical liver markers. None of the non-invasive tests performed well at distinguishing early from advanced fibrosis, emphasizing the need to refine these tests, including this analysis, to limit the use of the liver biopsy. Further work needs to be undertaken to better understand how the urine steroid metabolome changes over time in relation to liver phenotype.

Acknowledgements

This work was supported by the Wellcome Trust, the University of Oxford and Queen Mary University of London.