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Ability of soluble TREM2 and PRO-C3 as biomarkers to predict changes in MASLD activity

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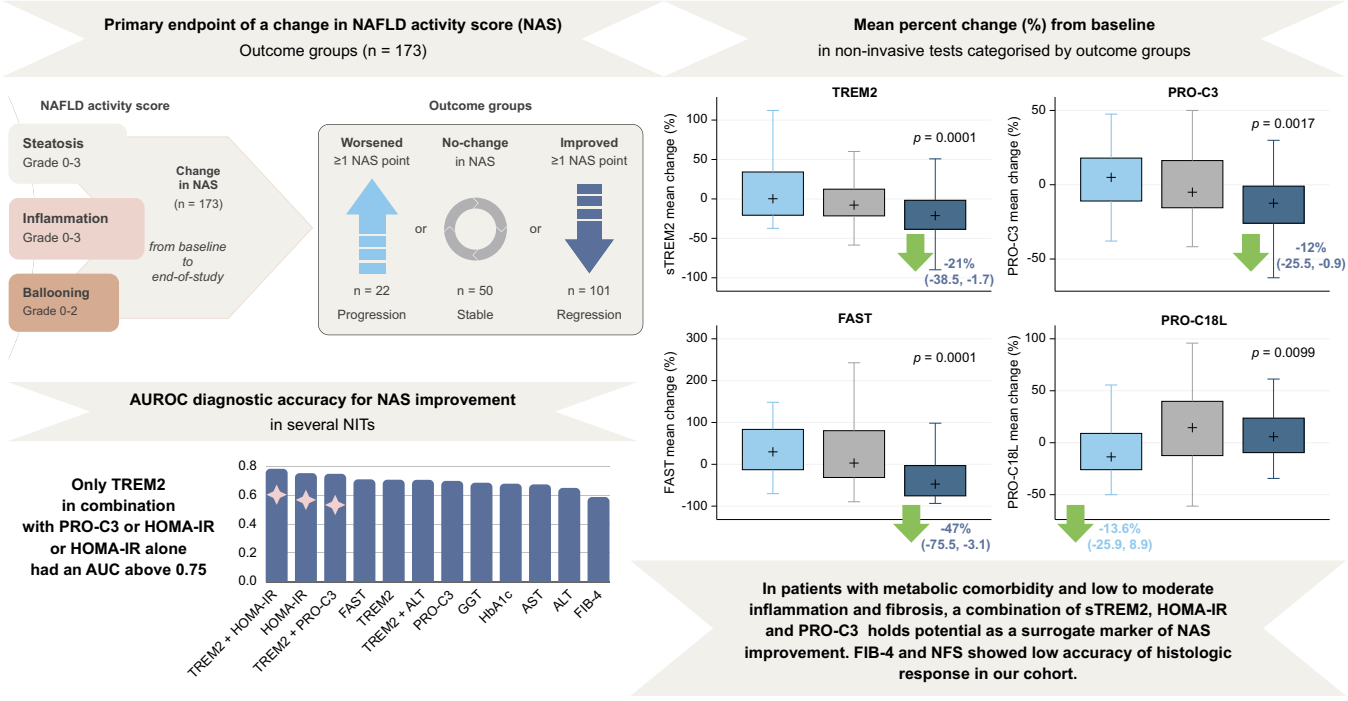
Graphical abstract



Highlights:

- NITs to accurately track and reflect treatment responses in patients with MASLD and MASH are an unmet need.
- This study presents a discovery and exploratory analysis of potential new biomarkers of treatment response.
- Patients who showed improvement in MASLD demonstrated lower levels of soluble TREM2, PRO-C3, FAST score, and HOMA-IR.
- The levels of a novel NIT, PRO-C18L, linked to basement membrane remodelling, were lower in patients whose disease worsened.

Impact and implications:

Non-invasive tests (NITs) will play a crucial role in monitoring treatment responses in metabolic dysfunction-associated steatotic liver disease, providing a viable alternative to liver biopsies. Our study investigates whether NITs reflect histological responses based on changes in the non-alcoholic fatty liver disease (NAFLD) activity score (NAS) in patients with type 2 diabetes mellitus or obesity. We used non-invasive markers, some corresponding to different biological aspects of disease severity. We found that reductions in certain NIT levels correlate well with NAS reduction and composite histological improvements (lobular inflammation and ballooning). Combining soluble triggering receptor expressed on myeloid cells 2, PRO-C3, or homeostatic model assessment of insulin resistance enhances the potential for monitoring NAS improvement.

https://doi.org/10.1016/j.jhepr.2025.101432

 JHEP Reports, 2025, 7, 1–13

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Ability of soluble TREM2 and PRO-C3 as biomarkers to predict changes in MASLD activity☆

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JHEP Reports **2025**. vol. 7 | 1–13

Background & Aims: Diet and weight loss remain the primary treatment for most patients with metabolic dysfunction-associated fatty liver disease (MASLD), with one recent drug therapy approved for severe cases. However, a significant need remains for non-invasive tests (NITs) that can assist clinicians in evaluating treatment response. We aimed to explore the ability of several NITs to reflect a change of at least one point in histologic non-alcoholic fatty liver disease (NAFLD) Activity Score (NAS).

Methods: This study explores biomarkers reflecting treatment response in 173 patients from secondary care with type 2 diabetes or severe obesity, all of whom underwent repeated liver biopsies and blood samples. We measured soluble triggering receptor expressed on myeloid cells 2 (TREM2), collagen markers PRO-C3, PRO-C4, PRO-C6, PRO-C8, and PRO-C18L and liver stiffness measured by FibroScan, FAST-score, and homeostatic model assessment of insulin resistance (HOMA-IR). We studied biomarker changes and their capacity to reflect liver biopsy alterations in two distinct cohorts, using comparative paired analyses and multivariable logistic regression to evaluate the results.

Results: Mean age was 52 years (±12), 38% male, 52% had NAS ≥3 at baseline (90/173), 70% had F0–F1 fibrosis, and 23% (39/173) had metabolic dysfunction-associated steatohepatitis. Significant differences were seen in sTREM2, PRO-C3, HOMA-IR, and FAST-score levels by NAS changes (worsened, no-change, improved) ($p = 0.0001$). In multivariable analysis, sTREM2 + PRO-C3 and HOMA-IR predicted NAS improvement (AUROC >0.75), with an odds ratio of 1.13 for each unit decrease ($p = 0.001$, 95% CI 1.04–1.21). FIB-4 and non-alcoholic fatty liver disease fibrosis score (NFS) did not reflect NAS improvement (AUROC <0.60, OR <1.05, p >0.5).

Conclusions: sTREM2, PRO-C3, and HOMA-IR indicate NAS improvement and warrant further investigation as surrogate markers for gauging intervention response.

Clinical Trials Registration: ClinicalTrials.gov (NCT03068078; NCT03535142).

Introduction

Metabolic dysfunction-associated steatohepatitis (MASH) is the progressive stage of metabolic dysfunction-associated steatotic liver disease (MASLD), characterised by hepatic steatosis, inflammation, hepatocyte damage, and fibrosis. Individuals with severe obesity or type 2 diabetes (T2DM) face a heightened risk of MASLD and developing MASH. Although liver biopsy remains the gold standard for diagnosing and monitoring MASH, practical constraints limit its accessibility.[1]

In early 2024, the FDA approved resmetirom for use in MASH, marking a significant milestone in the development of treatment.[2,3] The ESSENCE phase III trial of semaglutide also showed positive results,[4] and additional drugs are anticipated to yield results soon.[5] Despite these advancements, lifestyle intervention remains pivotal,[6] and metabolic surgeries like Roux-en-Y gastric bypass or sleeve gastrectomy are effective but limited to selected patients.[7]

Few studies have reported on the ability of non-invasive tests (NITs) to indicate treatment response. In the REGENERATE cohort, involving obeticholic acid, reductions were observed in Fibrosis-4 (FIB-4), aspartate aminotransferase (AST), and liver stiffness measurement (LSM) in patients with a one-stage fibrosis improvement.[8] Similarly, resmetirom phase II trial extensions showed reductions in liver fat, LSM, and the collagen III marker PRO-C3.[9]

However, these trials involved highly selected participants with MASH and varying fibrosis stages. There is a need for NITs to monitor disease changes, which are applicable to broader populations, including younger patients with less advanced disease.[10] One such marker is soluble triggering receptor expressed on myeloid cells 2 (sTREM2), which is shed from macrophages in

☆ Given their role as Associate Editors, Maja Thiele had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to the Co-Editor Jacob George and Associate Editor Jörn M. Schattenberg.

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https://doi.org/10.1016/j.jhepr.2025.101432

areas with hepatocellular damage, inflammation, and fibrosis.[11] This marker shows promise as a diagnostic test for MASH[11–13] and has been proposed as part of a composite diagnostic score.[14] We also assessed the neoepitope collagen marker PRO-C3,[15] a component of the ADAPT score,[16,17] and other collagen formation markers (PRO-C4, PRO-C6, PRO-C8).[18] Additionally, we explored PRO-C18L, associated with hepatocyte activity and basement membrane remodelling.[19] The primary aim of this study was to assess these NITs, individually or in combination, as monitoring tools for detecting at least a one-point change in histological NAFLD activity score (NAS).

Participants and methods

Study design and participants

This study is a discovery and exploratory analysis of biomarkers to reflect treatment response in MASLD. It included 173 participants with risk factors of MASLD and follow-up liver biopsy from two clinical cohorts previously reported in detail: (1) Odense cohort (n = 109), an RCT on dietary interventions in T2DM,[20] and (2) Esbjerg cohort (n = 64), a prospective case–control study in severe obesity (BMI ≥35 kg/m^2), with a subset undergoing bariatric surgery (Roux-en-Y gastric bypass or sleeve gastrectomy).[12] Not all had surgery, as some individuals opted out of the procedure, whereas others could not achieve the 8% weight loss required for surgery eligibility. Baseline visits preceded surgery, with a mean of 226 ± 121 days.

No alternative liver diseases were suspected or found, and participants were included based on risk factors (BMI for Esbjerg or T2DM for Odense), without prior non-invasive liver assessment. Following the ethical approvals for each clinical trial, all participants were offered liver biopsy at baseline and end-of-study (EOS) visits (Odense: 6 months; Esbjerg: ∼2.5 years). Recruitment and study visits were conducted at the University Hospitals of Odense and Esbjerg (2016–2022) (Table S1).

Participants were at least 18 years old and provided written informed consent. Exclusion criteria: other liver diseases, self-reported alcohol consumption >20 g/week (women) or >30 g/week (men), decompensated cirrhosis, use of hepatotoxic medications, pregnancy, or malignant diseases.

All investigations adhered to a standardised protocol during both visits. This included liver biopsy, medical history, transient elastography (TE) using FibroScan (Echosens, Paris, France), and blood sampling, all of which were conducted with data collection occurring on the same day under fasting conditions.

The trials complied with GCP standards and the Declaration of Helsinki. Ethical approval was obtained from regional ethics authorities: S-20150217 and S-20160006G. Data management utilised Redcap via the Open Patient Data Explorative Network (Odense, DK).

Non-invasive markers

Markers were assessed at baseline and EOS, with extra timepoints at 3 months post-EOS (Odense) and during surgery or control visit (Esbjerg).

Emerging markers

Plasma/serum samples were stored at -80 °C and shipped on dry ice for biomarker analysis. The external laboratories were blinded to clinical data during analysis. For a detailed description, see the Supplementary material and CTAT Table. Biomarker quantification followed the manufacturer's instructions.

For sTREM2, plasma levels were measured using the Human ELISA Kit (ab224881, Abcam) with acceptable CV% <10. No measurements fell below the lower detection limit.

Collagen formation neoepitope markers (collagen types III, IV, VI, VIII, XVIII-long) were measured by competitive ELISA according to Nordic Bioscience protocols; PRO-C3 (nordicPRO-C3^{TM}),[21] PRO-C4 (nordicPRO-C4^{TM}),[22] PRO-C6,[23] PRO-C8,[24] and PRO-C18 (technical paper under development); 1.7% to 1.1% of samples fell below the detection limit for PRO-C8 and PRO-C18L, and were assigned the lowest acceptable value. All plates were technically approved as per specifications.

Biochemical, TE, and composite markers

LSM and controlled attenuation parameter were measured by FibroScan. Experienced staff used M-/XL probes as indicated. A reliable measurement was defined as having at least 10 valid measurements and an IQR of <30% if the LSM was >7.1 kPa.[25]

Other NITs investigated included: ALT, aspartate transaminase (AST); gamma-glutamyl transferase (GGT), haemoglobin A1c (HbA1C); homeostatic model assessment of insulin resistance (HOMA-IR); FAST score; FIB-4; and NAFLD Fibrosis Score (NFS).

Assessment of liver biopsies

Percutaneous biopsies from the right liver lobe (16–18G suction needle) were analysed by two expert hepatopathologists (SD, Odense; TC, Esbjerg), blinded to all other data. Histology was graded using NAS (0–8) Clinical Research Network:[26] steatosis (0–3), lobular inflammation (0–3), and ballooning (0–2), along with a composite inflammatory activity score (inflammation and ballooning) (0–5), as described previously.[27] Fibrosis staging used the Kleiner fibrosis score (0–4). Biopsies were considered sufficient if ≥10 mm in length with six or more portal tracts or regenerative nodules; the mean biopsy length was 21.7 mm (SD 5.4).[28,29]

Outcomes

Patients were categorised based on NAS changes in liver biopsies between baseline and EOS: 'worsened by ≥1 NAS point,' 'no change in NAS,' and 'improved by ≥1 NAS point' (Fig. 1). This approach focused on liver biopsy results rather than treatment type (active *vs.* control) or cohort (Odense *vs.* Esbjerg). Biomarker changes were assessed in a combined cohort, with no FDA/EMA drug trial criteria applied, as pharmacological interventions were not used. We chose a change of 1 NAS point as the criterion because of the mild disease characteristics present in the study cohort, minimising the likelihood of more considerable changes (*e.g.* a 2-point change). The secondary aim was to assess changes of ≥1 point in both (1) activity composite score and (2) fibrosis stage. Changes in NITs from baseline were also calculated.

Statistics

Descriptive statistics are reported as mean ± SD, median (IQR), or frequencies. Between-group differences were tested using the X^2 test, Mann–Whitney *U* test, ANOVA, or Kruskal–Wallis test as appropriate. Logistic regression (n = 140 with

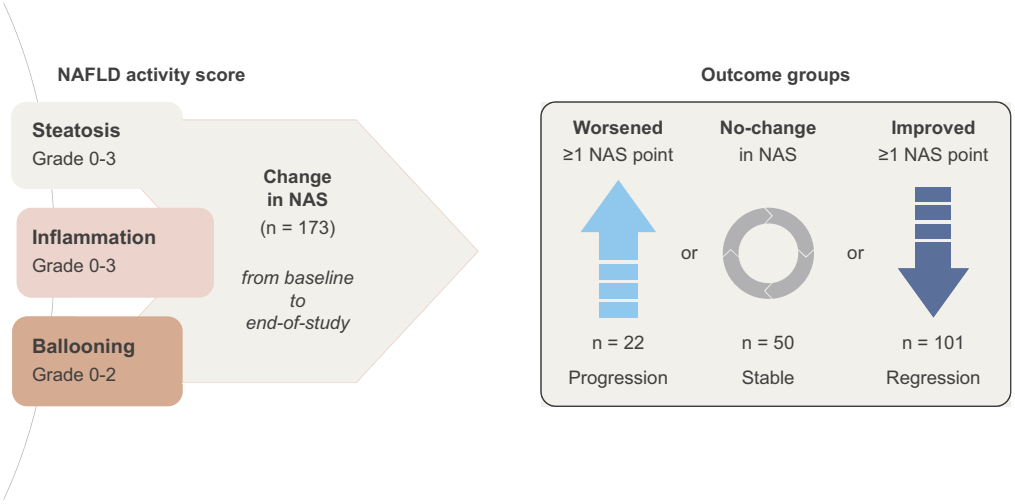


Fig. 1. Illustration of the primary endpoint: NAS change between baseline and EOS in 173 individuals. Groups: 'worsened' (≥1 NAS point, n = 22), 'no change' (n = 50), and 'improved' (≥1 NAS point, n = 101). EOS, end-of-study visit; NAS, NAFLD activity score.

complete data) assessed the probability of NAS improvement (≥1 point) *vs.* no improvement, reporting odds ratios (OR) with 95% confidence intervals and *p* values. An OR >1 indicates a decrease in a biomarker from baseline to EOS associated with higher odds of NAS improvement. Absolute changes in each NIT from baseline to EOT were included as unit differences in univariate and multivariate logistic regression models, adjusted for treatment (active/placebo), cohort (Odense/Esbjerg), weight change, ALT, and HOMA-IR. The ability of each NIT to predict NAS change was assessed using AUROC curves, with model fit evaluated by Akaike's Information Criterion (AIC). Values of $p < 0.05$ were considered significant. Analyses were conducted using Stata 17 (College Station, TX, USA) and R (version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

A total of 173 participants were included, and 19 did not have MASLD at baseline biopsy (NAS 0–1; no fibrosis, n = 11; F1, n = 7; F4, n = 1). We included all participants in the analysis because there was a possibility of histological change in at least one direction. The updated protocol restricts repeat biopsies in non-MASLD patients. The mean age was 52 (±12) years, comprising 38% males. T2DM was present in 73%, and 44% were using statins. At baseline, 70% had F0–F1 fibrosis, 29% had a NAS >4, and 23% had MASH; there was an equal prevalence of MASH of (21%; 25%) and NAS >4 (29%; 31%) between the Odense and Esbjerg cohorts but a somewhat different prevalence of advanced fibrosis F2–F4 (34%; 22%, $p = 0.002$). All baseline characteristics, separated by trial cohort (Odense and Esbjerg), are available in Table 1 (Fig. S1).

Outcome groups –-change in NAS between baseline and follow-up

Of the participants, 13% 'worsened', 29% had 'no change', and 58% 'improved' by ≥1 NAS point (n = 101). At baseline, participants in the 'improved' group were more metabolically sick with HOMA-IR of 7.7 ($p = 0.002$) and a higher prevalence of MASH ($p < 0.001$), see full characteristics in Table 2. The mean NAS change from baseline was similar between cohorts (Fig. S2A–C), but patients with at least one NAS point improvement showed a greater change in the Esbjerg cohort (-2.6 ± 1.6) compared to Odense (-1.6 ± 0.8). In the worsened group, 11 participants were from Odense (nine on a control diet, two on a low-carb, high-fat diet), and 11 were from Esbjerg, none of whom underwent bariatric surgery. Most participants had stable fibrosis (n = 102), 39 'worsened' by ≥1 stage, and 32 'improved' by ≥1 stage. The mean fibrosis change slightly increased in the Esbjerg cohort and remained stable in Odense (Fig. S2B).

Non-invasive tests and their response to changes in NAS

We examined the relationship between changes in NAS (baseline to EOS) and all NITs within the outcome groups. In patients who 'improved', several NITs showed significant decreases, including sTREM2, PRO-C3, HbA1c, HOMA-IR, LSM, CAP, and FAST-score ($p < 0.0001$), while PRO-C18L increased ($p = 0.02$). HbA1c slightly decreased ($p = 0.005$) in the 'no-change' group, and no other markers changed significantly. Among patients who 'worsened', PRO-C18 decreased, whereas PRO-C4 and HOMA-IR increased significantly ($p < 0.02$) (Table 3 and Table S2).

Weight change in patients who 'improved' had a mean weight loss of -12.0 kg (SD 14.70), while those in the 'no-change' group lost -9.00 kg (SD 18.05). In contrast, patients who 'worsened' had stable weight, with a mean change of +1.45 kg (SD 6.17) (Table 3 and Fig. S2C).

Emerging markers

In patients who 'improved' in NAS, sTREM2 was reduced in units by -7.1 (-19.2, -0.62) and PRO-C3 by -2.06 (-6.82; -0.18) (Table 3) and expressed as percentage, sTREM2 by -21.2% (-38.5, -1.7) and PRO-C3 by -12.4% (-25.4, -0.9) ($p < 0.001$; $p < 0.01$) (Fig. 2A and Table S4).

PRO-C18L, mainly produced by activated hepatocytes, showed an opposite pattern and decreased in patients who 'worsened' by 13.6% (-25.9, 8.9) ($p < 0.01$) (Fig. 2A and

Table S4). We observed that PRO-C4 increased in patients who 'worsened (p <0.02), but saw no significant changes for PRO-C6 or PRO-C8 (Table 3, Fig. 2B).

The decreased levels of sTREM2 and PRO-C3 exhibited a dose–response pattern, correlating with changes in NAS points of -1, -2, and <-2. However, these correlations plateaued for patients who experienced 'no-change' or 'worsened' NAS (Fig. 3).

Biochemistry markers

At the EOS visit, patients who showed improvement had significantly lower levels of several other NITs, reflecting this NAS change. These included HbA1c, HOMA-IR (Table 3), ALT, AST, and GGT (Table S2, Fig. S3B). HOMA-IR levels exhibited a step-wise change, closely mirroring NAS alterations, with apparent increases in patients who 'worsened' by 42.8% and decreases in patients who 'improved by 39.0% (p <0.001) (Fig. 2C).

The two composite NIT fibrosis scores, FIB-4 and NFS, did not change significantly from baseline, nor did these NITs reflect changes in NAS (p = 0.371) (Fig. 2B).

Imaging-based markers

A smaller sample was used for serial FibroScan measurements because 16% of the recordings were invalid. Nonetheless, we observed LSM changes ranging from -1.05 to 0.4 kPa across all outcome groups (Table 3). LSM values decreased in the 'improved' group by 17.0% (-33.3, 10.1) and in the 'worsened' group by 6.1% (-23.7, 10.3). The controlled attenuation parameter (dB/m), an indirect marker of liver steatosis, decreased by 12.1% (-24.1, 2.2) in the 'improved' group, with minor changes in the other groups (Fig. 2B and C). A notable reduction in the FAST score of 47.4% (-75.5, -3.1) was observed in the 'improved' NAS group compared with the others (p <0.001). The FAST score decreased and correlated with NAS changes of -1, -2, and <-2 points, but levelled off in patients with 'no change' or 'worsened' NAS (Fig. 3).

NITs in relation to changes in activity score and fibrosis stage

The downregulation of NITs observed in patients who 'improved' in NAS could be reflective of regression in steatosis. However, 70% of patients who 'improved' ≥1 NAS (70/101) had a change in the activity composite score grade (inflammation and ballooning). We stratified the cohort based on changes in the activity composite score. We could verify that sTREM2, PRO-C3, FAST, HbA1c, HOMA-IR, ALT, AST, GGT, LSM, and CAP followed the same pattern and direction of relative changes in the three outcome groups (improved, no-change, worsened), all with statistically significant differences between groups (p <0.01) (Table S3). We separately analysed TREM2, PRO-C3, PRO-C18L, and HOMA-IR in the improvement outcomes group for steatosis alone *vs.* activity (composite score), Fig. S5A and B. sTREM2 were significant in both steatosis and activity (p <0.001), PRO-C3 change was more strongly linked to activity improvement, and HOMA-IR to both steatosis and activity (p <0.001). PRO-C18L was significant for worsening steatosis and fibrosis but not activity (Table S3).

Thirty-two patients had at least a one-stage fibrosis improvement, and 69% (22/32) of these also 'improved' in NAS by ≥1 point. Only ALT, FAST-score, and PRO-C18L showed significant differences between groups with a one-stage change in fibrosis (Table S2). These changes followed the same pattern and direction of relative changes as seen in the box-plot figures for NAS change (Fig. 2A and Fig. S3). Fourteen percent of participants (24/173) could not improve in the fibrosis stage, as they had an F0 at baseline.

Table 1. Baseline characteristics by original trial cohort (n = 173).

	Esbjerg cohort (n = 64)	Odense cohort (n = 109)	Total (n = 173)	p value
Age, years	45.1 (12.8)	56.5 (9.7)	52.3 (12.2)	**<0.001**
Male sex, n (%)	21 (33)	45 (41)	66 (38)	0.268
BMI (kg/m^2)	43.6 (6.8)	35.0 (7.0)	38.1 (8.0)	**<0.001**
Type 2 diabetes* (yes), n (%)	17 (27)	109 (100)	126 (73)	**<0.001**
HOMA-IR†	6.0 (4.0–11.0)	6.8 (4.4–10.6)	6.5 (4.3–10.6)	0.422
Statins (yes), n (%)	17 (27)	60 (55)	77 (44.5)	**<0.001**
ALT (U/L)	31 (22–55)	31 (24–46)	31 (23–48)	0.549
AST (U/L)	25 (20–35)†	25 (20–32)†	25 (20–33)	0.752
GGT (U/L)	32 (24–57)†	33 (24–51)	33 (24–54)	0.968
LDL cholesterol (mmol/L)	3.0 (2.3–3.7)	2.2 (1.7–2.7)	2.5 (1.7–3.1)	**<0.001**
Triglycerides (mmol/L)	1.5 (1.0–2.0)	1.6 (1.2–1.5)	1.5 (1.1–2.2)	0.324
TREM2 (ng/ml)	37.4 (22–58)	38.4 (28–54)	38.0 (26–56)	0.921
Liver stiffness by TE (kPa)	7.9 (5.0–14.5)	5.9 (4.8–8.7)	6.2 (4.8–9.6)	0.026
Fibrosis, F0/F1/F2/F3/F4 (n)	17/33/9/3/2	8/64/33/3/1	25/97/42/6/3	**0.002**
NAS, 0-2, 3-5, 6-8, (n)	36/24/4	47/59/3	83/83/7	0.082
no-MASLD/MASLD/MASH, (n)	16/32/16	3/83/23	19/115/39	**<0.001**
GLP-1 (yes), n (%)	6 (9)	22 (20)	28 (16)	0.062
Long-acting insulin (yes), n (%)	4 (6)	14 (13)	18 (10)	0.170
Short-acting insulin (yes), n (%)	2 (3)	1 (1)	3 (2)	0.283
Metformin (yes), n (%)	10 (16)	93 (85)	103 (60)	**<0.001**

Categorical data as frequency (%), continuous data as mean (±SD) or median (IQR), depending on distribution. Differences between cohorts were assessed using the X^2 test or Mann–Whitney U test. Values of p in bold indicate statistical significance.

Missing values:

†=<5. ALT, alanine transaminase; AST, aspartate transaminase; Bariatric cohort, patients with severe obesity; BMI, Body Mass Index; GGT, gamma-glutamyl transferase; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; MASLD, metabolic dysfunction-associated fatty liver disease; MASH, metabolic dysfunction-associated steatohepatitis; NAS, NAFLD activity score; Statins, including other cholesterol-lowering medications; sTREM2, soluble triggering receptor expressed on myeloid cells; T2DM, type 2 diabetes.

*In the Bariatric cohort, T2DM patients were diagnosed or had a fasting glucose >6.9 mmol/L at baseline.

Table 2. Patient characteristics at baseline by histological NAS change at EOS: 'Worsened ≥1' (n = 22), 'No change' (n = 50), 'Improved ≥1' (n = 101).

	Worsened ≥1 NAS (n = 22)	Stable in NAS (n = 50)	Improved ≥1 NAS (n = 101)	*p* value
T2DM cohort, n (%)	11 (50)	30 (60)	68 (67)	0.272
Active intervention, n (%)	2 (9)	30 (60)	68 (67)	**<0.001**
Age, years	52 (38–57)	56 (45–62)	55 (49-59)	0.395
Sex, male, n (%)	7 (32)	24 (48)	35 (35)	0.228
BMI, kg/m^2	40 (8)	37 (7)	38 (8)	0.432
Type-2 diabetes, n (%)	13 (60)	33 (66)	80 (79)	0.064
LDL cholesterol (mmol/L)	2.7 (0.8)	2.4 (1.0)	2.5 (1.0)	0.435
HOMA-IR	6.3[†] (4.5-19.7)	4.6 (3.6–7.1)	7.7[†] (4.9-11.2)	**0.002**
Liver stiffness by TE, kPa	8.8 (5.7–14.0)	5.2 (4.1–7.9)	6.2 (5.3–10.8)	0.238
Statins* (yes), n (%)	8 (36)	20 (40)	49 (49)	0.436
GLP-1 (yes), n (%)	5 (23)	5 (10)	18 (18)	0.316
Long-acting insulin (yes), n (%)	4 (8)	4 (8)	10 (10)	0.414
Short-acting insulin (yes), n (%)	0 (0)	1 (2)	2 (2)	0.801
Metformin (yes), n (%)	11 (20)	250 (50)	67 (66)	0.097
Fibrosis stage, F0/F1/F2/F3/F4, n	3/14/4/1/0	13/23/10/2/2	9/60/28/3/1	0.276
NAFLD activity score, 0–2, 3–5, 6–8, n	12/10/0	40/10/0	31/63/7	**<0.001**
No-MASLD/MASLD/MASH, n	4/16/2	14/32/4	1/67/33	**<0.001**

Categorical data are presented as frequency (%), continuous data as mean (±SD) or median (IQR). Cohort differences were assessed using ANOVA or the Kruskal–Wallis test. Values of *p* in bold indicate statistical significance. Missing values:
[†]<5. BMI, Body Mass Index; EOS, end-of-study visit; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; MASLD, metabolic dysfunction-associated fatty liver disease; MASH, metabolic dysfunction-associated steatohepatitis; NAS, NAFLD activity scores; Statins, includes other cholesterol-lowering medications.

Biomarker dynamics across distinct study cohorts

Although the length of follow-up differs between the Odense and Esbjerg cohorts, the biomarker trajectories are shown to emphasise patterns within each cohort rather than for direct comparison (Fig. 4A and B and Fig. S4A–E).

We analysed each NIT's relative mean percentage changes (95% CI) across the three outcome groups. In the Esbjerg cohort, sTREM2 and PRO-C3 significantly decreased in patients who showed improvement in NAS, compared with other groups (p <0.001). The Odense cohort displayed a decline in sTREM for all groups, particularly in the 'improved' group, but no significant changes were noted in sTREM2 or PRO-C3 relative to baseline (p >0.05) (Fig. 4A).

In the Odense cohort, a decrease in PRO-C18L levels was seen in patients who worsened in NAS (p <0.05). In contrast, the Esbjerg cohort exhibited no significant changes (p >0.05) (Fig. 4B). Non-valid FAST score measurements were recorded at either visit for both Odense (n = 16) and Esbjerg (n = 12) cohorts; however, patients with improved NAS demonstrated a

Table 3. Biochemistry and markers at baseline, end-of-trial, and change by histologic NAS stage: 'Worsened ≥1' (n = 22), 'No change' (n = 50), and 'Improved ≥1' (n = 101).

Non-invasive test	n	Baseline, median (IQR)	End-of-study, median (IQR)	Changes in units from baseline to end-of-trial, median (IQR)	*p* value
Worsened ≥1 NAS score (n = 22)					
Emerging NIT of NASH and fibrosis					
TREM2 (ng/ml)	22	41.0 (27.2, 67.3)	40.7 (33.9, 65.7)	0.078 (-16.82, 9.34)	0.76
PRO-C3 (ng/ml)	22	23.8 (14.7, 26.4)	22.5 (15.8, 28.2)	1.27 (-3.4, 4.2)	0.45
PRO-C4 HP (ng/ml)	22	6,874 (5,822, 7,587)	7,633 (1,460, 8,363)	696 (15, 1,512)	**0.01**
PRO-C6 (ng/ml)	22	11.9 (9.8, 14.6)	12.0 (9.1, 14.1)	0.077 (-2.00, 1.38)	0.93
PRO-C8 (ng/ml)	22	3.08 (2.39, 4.10)	3.29 (2.35, 4.07)	-0.259 (-0.92, 0.56)	0.28
PRO-C18L (ng/ml)	22	9.1 (8.1, 12.9)	8.75 (6.8, 12.7)	-1.40 (-2.40, 0.60)	**0.02**
Composite fibrosis scores					
FIB-4	21	0.79 (0.67, 0.96)	0.84 (0.58, 1.01)	0.002 (-0.17, 0.18)	0.98
NFS	21	-1.20 (-1.80, 0.14)	-0.93 (-1.53, 0.41)	0.152 (-0.153, 0.429)	0.15
Biochemistry					
HbA1c (mmol/mol)	22	47 (37, 56)	49 (40, 59)	2.5 (-4, 4)	0.62
HOMA-IR (mmol/L)	19	6.3 (4.5, 19.7)	10.4 (6.2, 17.8)	2.2 (-0.3, 6.9)	**0.01**
Imaging-based markers					
LSM by TE, (kPa)	20	8.3 (5.7, 10.2)	7.5 (5.4, 9.6)	-0.35 (-2.20, 1,65)	0.52
CAP by TE, (db/m)	20	336 (307, 375)	355 (323, 378)	5 (-5, 28)	0.23
FAST	19	0.36 (0.08, 0.58)	0.3 (0.15, 0.66)	0.072 (-0.058, 0.117)	0.21
Liver biopsy					
Fibrosis stage (mean, SD)	22	1.13 (0.71)	1.50 (0.91)	0.36 (0.72)	**0.03**
Body composition					
Weight, kg (mean, SD)	22	120.4 (27.5)	121.9 (28.1)	1.45 (6.17)	0.14
Stable in NAS, no change (n = 50)					
Emerging NIT of NASH and fibrosis					
TREM2 (ng/ml)	50	30.8 (22.3, 43.9)	28.9 (20.4, 36.5)	-1.926 (-6.57, 2.24)	0.13
PRO-C3 (ng/ml)	50	21.4 (14.8, 25.5)	18.6 (15.0, 26.1)	-0.75 (-3.37, 2.98)	0.84

(*continued on next page*)

Table 3. (*continued*)

Non-invasive test	n	Baseline, median (IQR)	End-of-study, median (IQR)	Changes in units from baseline to end-of-trial, median (IQR)	*p* value
PRO-C4 (ng/ml)	50	6,134 (5,593, 6,797)	6,715 (2,247, 7,760)	395 (-325, 1,334)	0.06
PRO-C6 (ng/ml)	50	10.7 (9.0, 13.3)	12 (9.4, 15.6)	0.20 (-1.10, 2.26)	0.27
PRO-C8 (ng/ml)	50	2.84 (2.42, 3.71)	2.92 (1.40, 2.26)	0.040 (-0.37, 0.78)	0.58
PRO-C18L (ng/ml)	50	10.3 (6.6, 14.7)	11.1 (8.3, 14.9)	1.10 (-1.20, 3.20)	0.06
Composite fibrosis scores					
FIB-4	49	0.88 (0.61, 1.44)	1.02 (0.75, 1.54)	0.078 (-0.10, 0.27)	0.05
NFS	49	-0.47 (-1.23, 0.37)	-0.33(-1.54, 0.24)	-0.030 (-0.482, 0.350)	0.51
Routine biochemistry					
HbA1c (mmol/mol)	49	50 (36, 54)	44 (35, 52)	-2 (-11, 1)	**0.005**
HOMA-IR (mmol/L)	50	4.6 (3.6, 7.1)	3.9 (2.4, 9.3)	-1.1 (-2.2, 1.1)	0.23
Imaging-based markers					
LSM by TE (kPa)	45	5.2 (4.1, 7.9)	5.7 (4.7, 8.6)	0.4 (-0.70, 1.50)	0.13
CAP by TE (db/m)	45	307 (268, 338)	288 (252, 341)	-2 (-42, 21)	0.58
FAST	42	0.13 (0.05, 0.29)	0.17 (0.06, 0.27)	0.005 (-0.043, 0.066)	0.36
Liver biopsy					
Fibrosis stage (mean, SD)	50	1.14 (0.99)	1.22 (0.93)	0.08 (0.63)	0.37
Body composition					
Weight, kg (mean, SD)	50	109.7 (23.5)	100.7 (23.0)	-9.00 (18.05)	<0.001
Improved ≥1 NAS score (n = 101)					
Emerging NIT of NASH and fibrosis					
sTREM2 (ng/ml)	101	42.8 (28.1-64.8)	31.5 (23.4, 39.8)	-7.070 (-19.12, -0.62)	**<0.0001**
PRO-C3 (ng/ml)	101	18.2 (15.3, 26.5)	16.2 (13.2, 22.9)	-2.06 (-6.82, -0.18)	**<0.0001**
PRO-C4 (ng/ml)	101	6,600 (5,845, 7,366)	6,911 (1,849, 7,903)	196 (-565, 1,035)	0.06
PRO-C6 (ng/ml)	101	10.3 (9.1, 12.7)	10.2 (8.85, 11.95)	-0.204 (-1.90, 0.23)	0.42
PRO-C8 (ng/ml)	101	3.25 (2.19, 4.70)	2.94 (2.19, 4.16)	-0.100 (-0.65, 0.28)	0.06
PRO-C18L (ng/ml)	101	8.9 (7.0, 12.0)	9.5 (7.0, 13.2)	0.60 (-0.80, 2.2)	**0.02**
Composite NIT of fibrosis					
FIB-4	91	0.90 (0.63, 1.22)	0.87 (0.70, 1.26)	0.048 (-0.17, 0.20)	0.41
NFS	97	-0.74 (-1.56, 0.17)	-0.95 (-1.67, 0.19)	-0.029 (-0.340, 0.357)	0.66
Biochemistry					
HbA1c (mmol/mol)	101	52 (45, 58)	44 (35, 52)	-7 (-12, -3)	**<0.0001**
HOMA-IR (mmol/L)	97	7.7 (4.9, 11.2)	4.5 (2.3, 7.2)	-2.5 (-5.9, -0.5)	**<0.0001**
Imaging-based markers					
LSM by TE, (kPa)	94	6.1 (5.2, 9.3)	5.4 (4.3, 8.1)	-1.05 (-2.7, 0.70)	**0.0001**
CAP by TE, (db/m)	94	350 (307, 386)	311 (254, 338)	-46 (-83, 8)	**<0.0001**
FAST	84	0.32 (0.13, 0.60)	0.13 (0.06, 0.25)	-0.090 (-0.299, -0.002)	**<0.0001**
Liver biopsy					
Fibrosis stage (mean, SD)	101	1.28 (0.71)	1.22 (0.81)	-0.05 (0.65)	0.50
Body composition					
Weight, kg (mean, SD)		111.6 (27.0)	99.5 (23.5)	-12.01 (14.70)	**<0.0001**

End-of-trial biopsies were taken 6 months (Odense) or 30 months (Esbjerg) after baseline. Data are presented as medians (IQR) unless otherwise indicated. Statistical analysis: Wilcoxon matched-pairs signed-rank test, with outliers included. Values of *p* in bold indicate significance. CAP by TE, Controlled Attenuation Parameter by transient elastography; FAST, FibroScan-AST; FIB-4, fibrosis-4 score; Fibrosis, Kleiner fibrosis score; HbA1c, haemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance; LSM by TE, liver stiffness by transient elastography; NASH, non-alcoholic steatohepatitis; NFS, NAFLD fibrosis score; NIT, non-invasive test; PRO-C3-C8, collagen pro-peptides (III, IV, VI, VII); PRO-C18L, basement membrane collagen (XVIII); sTREM2, soluble triggering receptor expressed on myeloid cells.

significant decrease in FAST score across both cohorts (p <0.01), correlating with fibrosis changes (Table S3). The biomarkers ALT, AST, HOMA-IR, HbA1c, and GGT produced similar results in both outcome groups of the Odense and Esbjerg cohorts (Fig. 4A–E). Notably, HOMA-IR and GGT displayed the most defined separation from the other two outcome groups in both Odense (p <0.01) and Esbjerg cohorts (p <0.001), unlike ALT, AST, and HbA1c, which showed greater overlap (Fig. 4A–E).

Additionally, body weight changes for patients who improved in NAS differed between cohorts, with a median decrease of 6.5 kg (SD 5.4) in Odense and of 23 kg (SD 20) in Esbjerg (Fig. 2C).

Logistic regression and composite scores for response

All logistic regression models accounted for treatment arm (active/intervention *vs.* placebo/none) and cohort heterogeneity (Odense *vs.* Esbjerg). Univariate logistic regression models identified significant odds ratios (OR >1, p <0.05), indicating statistical significance. Multivariable logistic regression showed that changes in TREM2 and PRO-C3 remained significant. After adjusting for weight, ALT, and HOMA-IR (Table S5), sTREM2 and PRO-C3 changes continued to be significant, suggesting these biomarkers may help assess histological change. However, NAS improvement showed a relatively weak association with most NITs, as evidenced by an AUROC <0.7 (Fig. 5). Nevertheless, the AUROC for HOMA-IR (0.76), FAST (0.71), sTREM2 (0.71), and PRO-C3 (0.70) indicated reasonable discrimination, which improved in multivariable models incorporating multiple NITs, such as sTREM2 + HOMA-IR (AUROC 0.79) and sTREM2 + PRO-C3 (AUROC 0.75) (Fig. 5A and C).

Discussion

Monitoring response to treatment or changes in lifestyle in patients at risk of MASLD and MASH is an unmet clinical need. We believe that the biological attributes of markers are critical

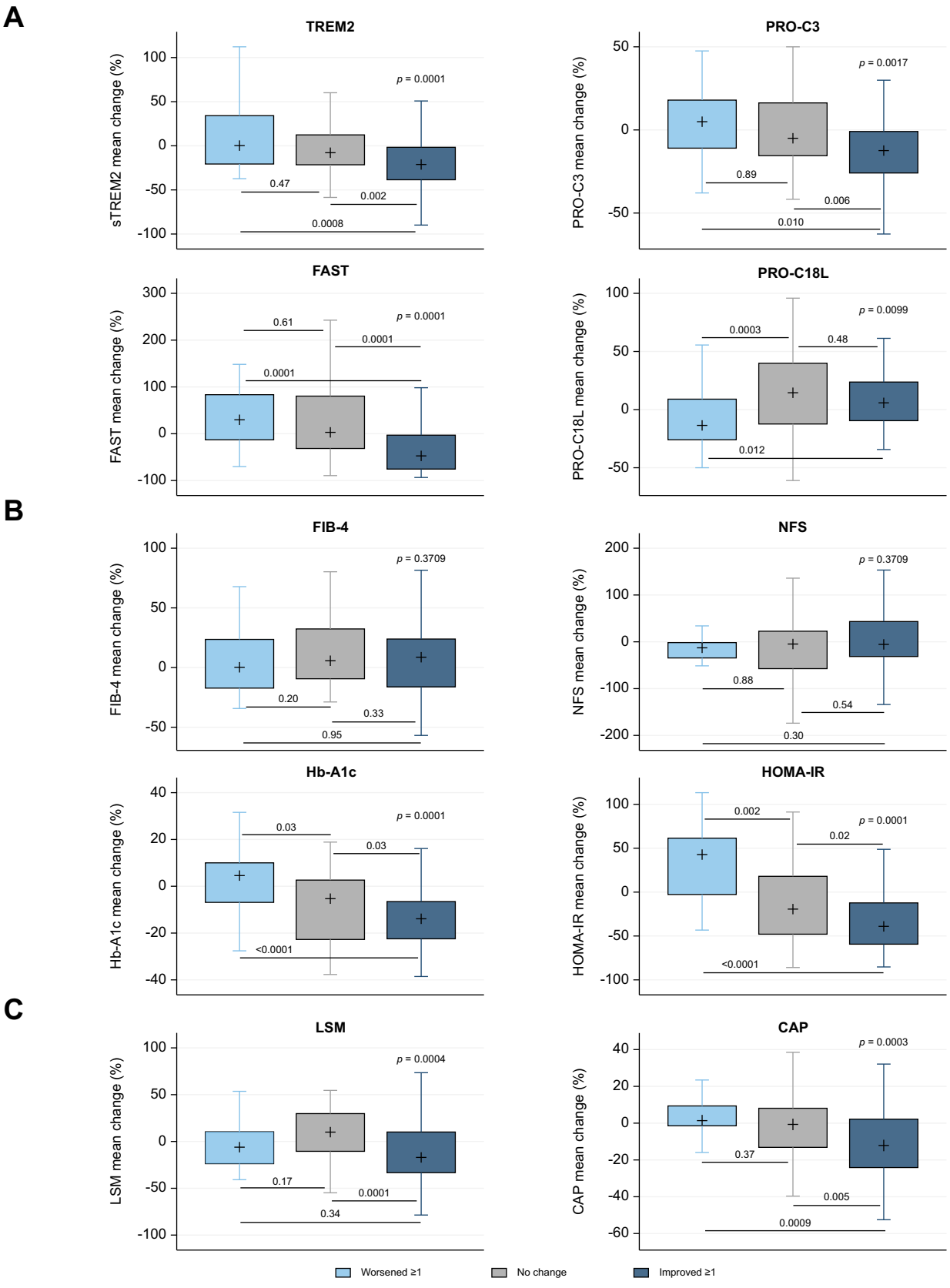


Fig. 2. Relative percent changes in markers at EOS by NAS change in several markers. (A–C) Relative percent changes in markers at EOS by NAS change: worsened (light blue, n = 22), no change (grey, n = 50), improved (navy, n = 101). Median (black +), IQR (boxes). Outliers were included in statistical tests but removed from the figure. Statistical test: Kruskal–Wallis paired rank, Bonferroni correction. Y-axes have different scales. CAP, controlled attenuation parameter (FibroScan); EOS, end-of-study visit; FAST, FibroScan-AST score; FIB-4, fibrosis-4 score; HbA1c, haemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin resistance; LSM by TE, liver stiffness

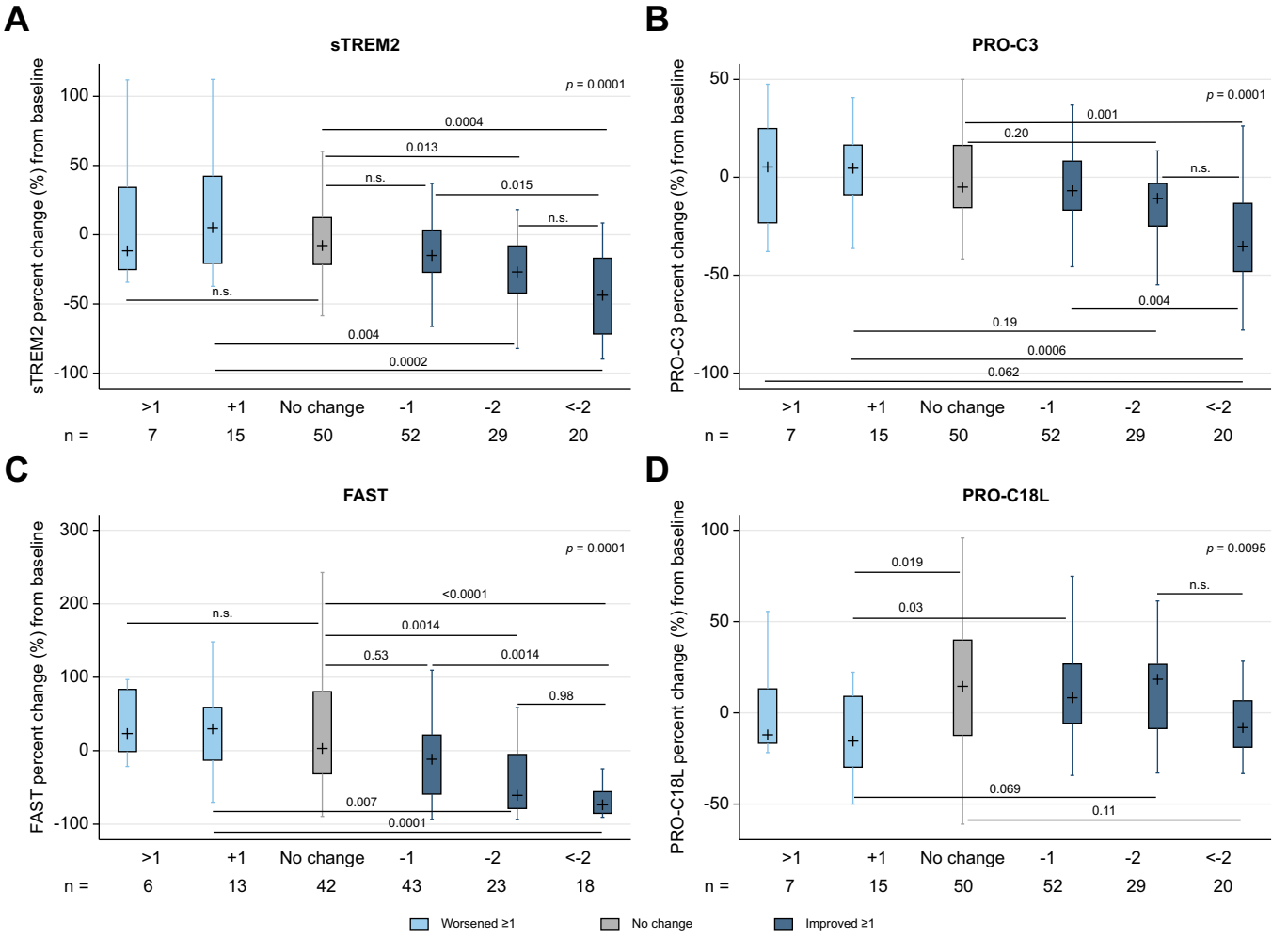


Fig. 3. Relative percent changes in sTREM2, PRO-C3, FAST, and PRO-C18L at EOS by six NAS change levels: worsened (>+1, +1), no change, improved (-1, -2, <-2). Statistical test: Kruskal–Wallis, Bonferroni correction. Median (black +), IQR (box). Y-axes have different scales. Outliers were included in the analysis but not shown. Y-axes have different scales. EOS, end-of-study visit; FAST, FibroScan-AST; NAS, NAFLD activity score; PRO-C18L, basement membrane collagen type XVIII; PRO-C3, collagen type III; sTREM2, soluble, triggering receptor expressed on myeloid cells type 2.

for their viability as surrogate endpoints for histological changes. Our study showed that regardless of treatment, patients who 'improved' at least one NAS point, of which 70% also 'improved' in either inflammation or ballooning (compared with patients who were stable or progressed), had pronounced reduction in several NITs, including sTREM, PRO-C3, HOMA-IR, and FAST. These markers showed a dose–response relationship. Combining sTREM2 with either PRO-C3 or HOMA-IR in multivariable regression models could confidently predict NAS improvement with an AUROC >0.75.

PRO-C3 is a validated marker of collagen formation, fibroblast activation, and disease activity[15,16] and our study could verify findings from the Resmetirom study concerning reduced levels of PRO-C3 when the NAS score improves.[9] sTREM2, although more recently described, has been localised by spatial transcriptomics to areas of hepatocellular damage and fibrosis in the liver[11] and performs well as a diagnostic marker for MASH.[12] This is the first study where sTREM2 is serially measured to investigate the marker's ability to reflect the inflammatory change.

HOMA-IR showed a distinct dose–response linked to NAS changes, reflecting both regression and worsening. This aligns with its role in MASH progression, including systemic insulin resistance and inflammation.[30] Although HOMA-IR is evaluated as a diagnostic marker for MASH with reasonable accuracy,[31] it has a low prognostic value.[32] In this cohort, HOMA-IR shows potential as a monitoring marker, but variability in assay methods, intra-individual differences, and fluctuations with antidiabetic medications[33] may hinder reliable tracking of longitudinal changes.

The FAST score, a composite marker of liver tissue assessed via elastography and AST, showed significant dose–

measurement by transient elastography (FibroScan); NAS, NAFLD activity score; NFS, NAFLD fibrosis score; PRO-C18L, basement membrane collagen type XVIII; PRO-C3, collagen type III; sTREM2, soluble, triggering receptor expressed on myeloid cells type 2.

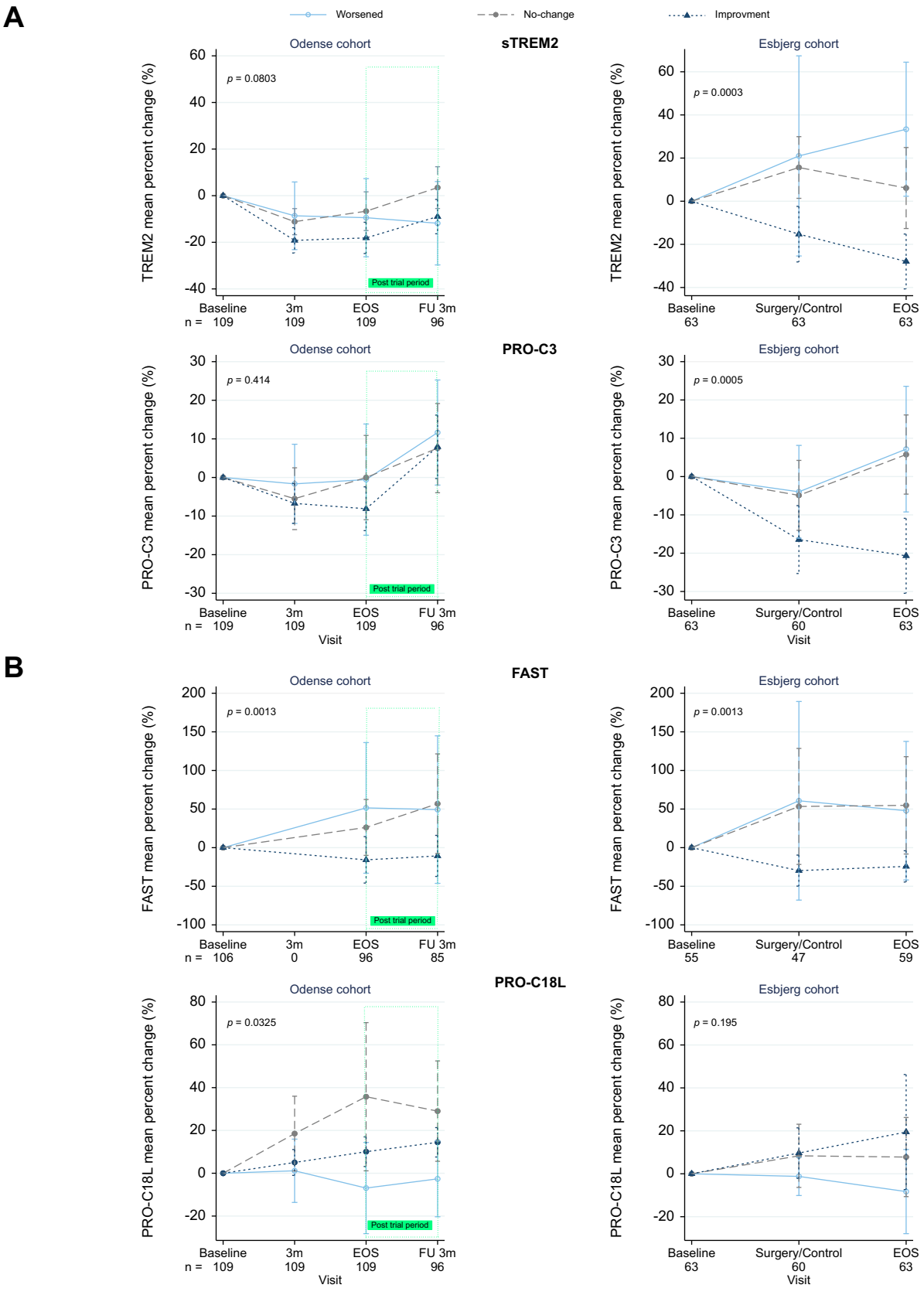
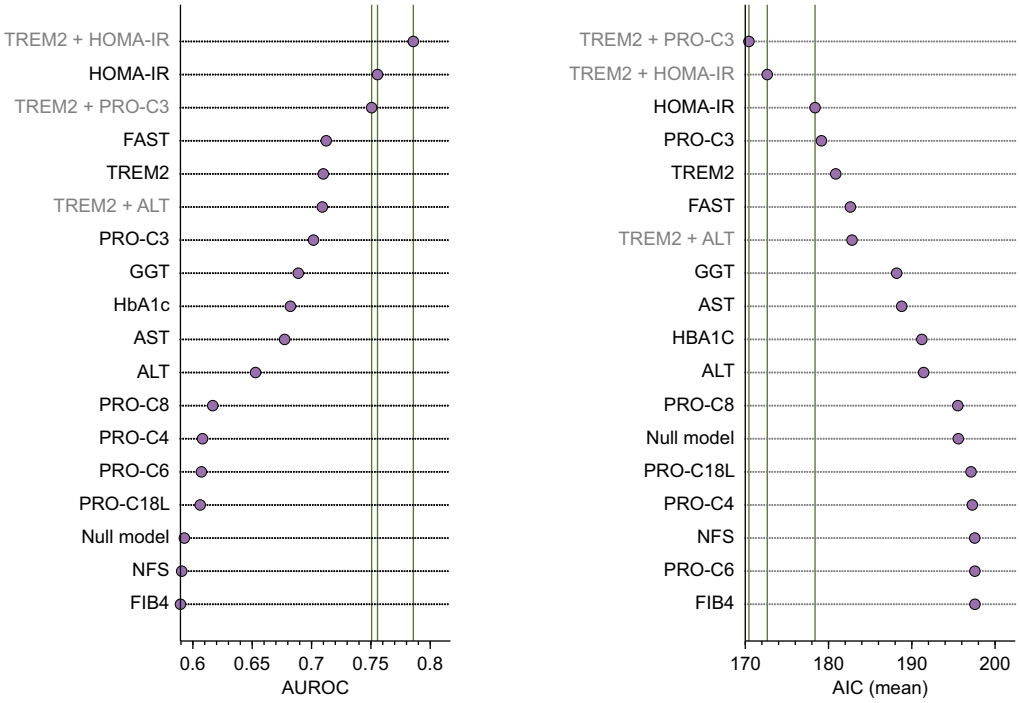


Fig. 4. Relative mean percent change of markers by cohort and outcome groups. (A, B) Relative mean percent change of markers by cohort and outcome groups. Statistical test: Kruskal–Wallis rank paired test. Mean (95% CI). Cases had surgery, controls had no intervention (Esbjerg). (A) TREM2, Triggering Receptor Expressed on Myeloid cells type 2; and PRO-C18L, pericellular basement membrane remodeling collagen type XVIII. (B) FAST score and PRO-C18L over time according to histologic NAS change at EOS (worsened ≥1 point, no-change, and improved ≥1 point). 3m, 3 months; EOS, end-of-study; FAST, FibroScan-AST; FU 3m, follow-up after 3 months; PRO-C18L, basement membrane collagen type XVIII.

A



B

Multivariable	Variable	OR	*p* value	95% CI
sTREM2 + HOMA-IR	TREM2	1.037	0.019	1.01-1.07
	HOMA-IR	1.173	0.007	1.04-1.32
sTREM2 + PRO-C3	TREM2	1.050	0.003	1.02-1.09
	PRO-C3	1.128	0.001	1.05-1.21
sTREM2 + ALT	TREM2	1.047	0.006	1.01-1.08
	ALT	1.003	0.83	0.97-1.03

C

Univariable	OR	*p* value	95% CI
sTREM2	1.049	0.001	1.02-1.08
PRO-C18L	1.014	0.50	0.97-1.06
PRO-C3	1.037	<0.001	1.02-1.06
PRO-C4	1.000	0.57	0.99-1.00
PRO-C6	1.005	0.91	0.92-1.09
PRO-C8	1.207	0.172	0.92-1.58
HbA1c	1.043	0.022	1.00-1.08
HOMA-IR	1.224	<0.001	1.09-1.37
FIB-4	1.024	0.96	0.42-2.48
NFS	1.043	0.87	0.64-1.71
AST	1.043	0.010	1.01-1.08
ALT	1.025	0.024	1.00-1.05
GGT	1.020	0.019	1.00-1.03
**FAST	48.3	<0.001	5.1-456.4

Fig. 5. Logistic regression predicting NAS improvement. (A–C) Logistic regression predicting NAS improvement (≥1 point, coded as 1) *vs.* no improvement (coded as 0). The models include treatment groups (active *vs.* placebo), the study cohorts, and unit changes in NITs. (A) AUROC and AIC model prediction. (B) Odds ratio for univariable models. **The FAST score shows a limited range (min: -0.78 and max: 0.65) compared with other NITs. (C) Multivariable logistic regression models. *Model based on n = 140 with complete data. AIC, Akaike's information criterion; FAST, FibroScan-AST; NAS, NAFLD activity score; NIT, non-invasive test.

response changes in our study, reflecting NAS improvement. This is in line with findings from the REGENERATE cohort in patients receiving obeticholic acid.[8] However, 16% of measurements were unreliable in our study, likely as a result of abdominal obesity, a challenge even with the XL probe.[34]

We also measured PRO-C18L, a novel biomarker reflecting levels of the long isotype of type XVIII collagen, known to be predominantly expressed in liver tissue and a member of the basement membrane family.[19] Preclinical studies have demonstrated that type XVII collagen constitutes a vital functional component within the liver matrix microenvironment, playing a crucial role in supporting hepatocyte survival during injury and stress[35] as observed during inflammation and fibrogenesis. We found lower levels of PRO-C18L in patients who 'worsened' in NAS in the overall cohort, driven by the Odense cohort. As the basement membrane serves as the scaffolding for hepatocytes, and considering the crucial role of hepatocytes as essential sources of type XVIII collagen,[19,35] a decrease in hepatocyte signalling for regeneration becomes a plausible observation. Knowledge of type XVIII collagen function fits well with our results, where decreased levels are seen in patients with a progression in

NAS. This highlights PRO-C18L as an interesting marker. The clinical implications of reduced levels of PRO-C18L could be that the regenerative function of the hepatocytes is impaired, leading to more severe disease.

We observed no significant changes in FIB-4 across different outcome groups regarding NAS change, composite inflammatory activity, or fibrosis stage. This contrasts with findings from the REGENERATE study, where the FIB-4 score was lower in patients showing at least a one-point improvement in fibrosis stage.[8] This discrepancy may stem from differences in cohort characteristics and the baseline prevalence of fibrosis severity, as over 50% of the REGENERATE cohort had advanced fibrosis (F3). Conversely, our cohort exhibited a low prevalence of advanced fibrosis at only 5%. Additionally, in our study, 14% (25/173) had no fibrosis at baseline, and thus could not improve in fibrosis. However, FIB-4 was primarily developed as a diagnostic score for fibrosis, and not as a monitoring marker. The inclusion of markers that may better predict NAS changes, such as CK18[36] and NIS,[37] will be interesting and important to investigate in a monitoring context.

This study focuses exclusively on the changes in biomarkers related to lifestyle and bariatric surgery interventions. Our analysis centres on measuring biomarkers repeatedly to evaluate disease status over time. Unlike predictive or prognostic biomarkers, which aim to anticipate future disease progression, monitoring biomarkers reflect current changes in disease state or treatment response.[1] The impact of drug therapy on biomarker levels is yet to be determined, as these drugs can influence biomarker levels based on their mechanisms of action. Although we have noted differences between the two cohorts, it is important to recognise that variations in the treatments investigated and follow-up periods (6 months for Odense and 2.5 years for Esbjerg) might influence the differences observed.

A primary distinction between the two groups was that some patients in the Esbjerg cohort who showed 'improvement' in NAS had a more pronounced change, likely attributable to the well-established impacts of bariatric surgery. Conversely, antidiabetic medications were more commonly utilised in the Odense cohort, particularly GLP-1 analogues (20% *vs.* 9%) and metformin (85% *vs.* 16%). GLP-1 analogues are known to promote weight loss and lower the density of inflammatory macrophages[38] whereas metformin may reduce proinflammatory cytokines, which could affect biomarker levels.[39,40] Notably, at least 25% of severely obese patients in the Esbjerg cohort had diabetes, and a greater proportion was identified as prediabetic. We saw strong associations in the Esbjerg cohort for biomarkers such as sTREM2 and PRO-C3. This variation may be attributed to the extended follow-up period in this cohort. Also, differences in patient characteristics, such as a higher prevalence of comorbidities and older age in the Odense cohort, possibly indicate a longer history of MASLD or MASH. Nonetheless, these explanations are still speculative. We should also take into account the previous limitation mentioned, as we did not evaluate other specific inflammatory markers such as interleukins, adiponectin, or leptin that might have offered additional insights.

A limitation is the lack of a more prognostic outcome, such as MASH resolution; investigating this would have required substantially reducing the sample size. However, it is crucial to recognise that the patients in our study reflect real-world populations affected by MASLD and MASH. Drug-development cohorts typically feature highly selective populations, contrasting with the demographics of our study group, which must be factored in when interpreting our results. The lack of consensus histology reading is also a limitation; but the paired design reduces interobserver variability. Despite the complexities associated with the heterogeneous cohort, markers such as HOMA-IR, sTREM2, PRO-C3, and PRO-C18L showed significant and promising outcomes. This research marks a progressive step toward identifying potential monitoring markers for future validation studies.

Conclusions

This exploratory analysis identified potential biomarkers for monitoring MASLD activity and treatment response. In patients with metabolic comorbidity and low to moderate inflammation and fibrosis, sTREM2 combined with PRO-C3 or HOMA-IR or HOMA-IR, in combination with other NITs, may reflect NAS improvement and has potential as a monitoring marker. PRO-C18L is also promising and warrants further investigation. FIB-4 and NFS had limited accuracy in detecting fibrosis response. More extensive studies with longer follow-ups, aligned to EMA and FDA standards, are needed to validate these findings, study fibrosis regression, and investigate biomarker responses across MASLD subtypes.

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Abbreviations

AIC, Akaike's information criterion; ALT, alanine transaminase; ANOVA, Analysis of Variance; AST, aspartate transaminase; AUROC, area under the receiver-operating characteristic curve; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; EMA, the European Medicines Agency; EOS, end-of-study visit; FAST-score, Fibroscan-AST score; FIB-4, fibrosis-4; FDA, food and drug administration; GCP, good clinical practice; GGT, gamma-glutamyl transferase; HbA1c, haemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance; LSM, liver stiffness measurement; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated fatty liver disease; NAS, NAFLD activity score; NFS, NAFLD fibrosis score; NIT, non-invasive test; OR, odds ratio; PRO-C3, -C4, -C6, -C8, and -C18L, collagen markers type III, IV, VI, VIII, XVIII long; RCT, randomised controlled trial; sTREM2, soluble triggering receptor expressed on myeloid cells 2; T2DM, type 2 diabetes mellitus; TE, transient elastography.

Financial support

The work was supported by the Danish National Research Foundation (grant DNRF141) to the Center for Functional Genomics and Tissue Plasticity (ATLAS). Region of Southern Denmark PhD salary grant and project grant (19/12166) (CW), by the Danish Council for Independent Research under Grant 4004-001 (JG) and Novo

Nordisk Foundation through two grants (NNF16OC0021038 (AK, CH) and NN23OC0086062 (JG)).

Conflicts of interest

DJL, AMG, and MK are all full-time employees at Nordic Bioscience A/S. DJL and MK are stockholders of Nordic Bioscience A/S. MT has received speaking fees from Siemens Healthcare, Norgine, Echosens, and Tillotts Pharma, consulting fees from GE Healthcare, Boehringer Ingelheim, and GSK, and is vice chair on the board of Alcohol & Society (NGO) and co-founder and board member of Evido. AK has served as a speaker for Novo Nordisk, Norgine, Siemens and Nordic Bioscience and participated in advisory boards for Norgine, Siemens, Resalis Therapeutics, Boehringer Ingelheim and Novo Nordisk, all outside the submitted work. Research support: Norgine, Siemens, Nordic Bioscience, AstraZeneca, Echosens. Board member and co-founder of Evido. All other authors declare no conflicts of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Concept and design: CW, VIC, JHG, MT, MK, AK. Experiments and data curation: VIC, MKS, TC, SD, MK, DJL, AMG. Investigation and data curation: CW, MML, CDH, LLG, BG. Writing – original draft: CW, IFV, MML, MST, AK. Writing – review and editing: all authors. Data analysis, visualisation, and formal analysis: CW. Funding acquisition: CW, CDH, MML, JHG, MK, DJL, AK.

Data availability statement

Because of data protection laws, the datasets created and analysed in this study cannot be accessed publicly. However, data from the Danish cohorts can be obtained in a pseudonymised format with approval from the Danish Data Protection Agency. Access requests should be sent to the corresponding author (AK) at aleksander.krag@rsyd.dk. The study protocol and statistical analysis plan remain unavailable.

Acknowledgements

We are grateful for the help from Gunhild Brixen, Medical Laboratory Technologist (Esbjerg). We thank academic collaborators Thomas W. Møller at Nordic Bioscience for extensive laboratory work with the collagen markers, Klaus Kaae Andersen and Gašper Letnar at Omicron for statistical advice, and Jude Pedersen for language editing. Thanks to the people at the Gastroenterology Department (Odense and Esbjerg) and FLASH (Odense University Hospital) who have contributed to the work.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2025.101432.

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Author names in bold designate shared co-first authorship

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Keywords: Serial liver biopsy; Hepatic inflammation; Neoepitope biomarkers; Collagen markers; Non-alcoholic steatohepatitis; Monitoring marker; Response marker.
Received 5 January 2025; received in revised form 10 April 2025; accepted 17 April 2025; Available online 22 April 2025