FibroScan-aspartate aminotransferase (FAST) score for monitoring histological improvement in non-alcoholic steatohepatitis activity during semaglutide treatment: post-hoc analysis of a randomised, double-blind, placebo-controlled, phase 2b trial

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Summary

Background Currently, assessment of candidate pharmacotherapies in patients with non-alcoholic steatohepatitis (NASH) involves invasive liver biopsy. Non-invasive scores, such as the FibroScan-aspartate aminotransferase (FAST) score, are used to identify candidates for therapy, but their ability to assess disease progression or treatment effect is unknown. We aimed to assess the association between FAST score and histological endpoints.

Methods We conducted a post-hoc analysis using data from a prior randomised, double-blind, placebo-controlled, phase 2b trial at 143 sites across 16 countries. Patients (aged 18–75 years) with biopsy-confirmed NASH, fibrosis stage 1–3, and a Non-alcoholic fatty liver disease Activity Score (NAS) ≥4 were enrolled between January 2017 and September 2018, and randomly assigned to receive once-daily subcutaneous semaglutide 0.1, 0.2, or 0.4 mg or placebo for 72 weeks. A subgroup analysis of patients with FAST score and histological data in the pooled semaglutide treatment and placebo arms at baseline and week 72 was performed. The original trial is registered at ClinicalTrials.gov, NCT02970942.

Findings A total of 122 patients were included in this post-hoc analysis (93 received semaglutide and 29 received placebo). FAST score reduction was associated with achieving the primary endpoint of NASH resolution without worsening of fibrosis in the pooled semaglutide group (area under the receiver operating curve 0.69; 95% confidence interval [CI] 0.58, 0.81). Mean FAST score reduction from baseline to week 72 was greatest in patients who met the primary endpoint *vs* those who did not in both the semaglutide (–0.40 [95% CI –0.84, 0.04] *vs* –0.22 [95% CI –0.74, 0.30] points; p = 0.002) and placebo groups (–0.25 [95% CI –0.72, 0.23] *vs* 0.00 [95% CI –0.50, 0.50] points; p = 0.047). Similarly, mean reductions in FAST score at week 72 were greater in those with NAS improvement *vs* those without in the semaglutide and placebo groups (≥1 point, –0.36 [95% CI –0.82, 0.11] *vs* –0.08 [95% CI –0.53, 0.38] points [p < 0.001] and –0.25 [95% CI –0.64, 0.14] *vs* –0.06 [95% CI –0.40, 0.53] points [p = 0.001]; ≥2 points, –0.40 [95% CI –0.86, 0.06] *vs* –0.14 [95% CI –0.56, 0.28] points [p < 0.001] and –0.29 [95% CI –0.67, 0.09] *vs* –0.05 [95% CI –0.40, 0.50] points; [p < 0.001]). A FAST score reduction of more than 0.22 points after semaglutide treatment was associated with meeting the primary endpoint (sensitivity 78%; specificity 60%; positive likelihood ratio 1.26; negative likelihood ratio 0.25; odds ratio 4.93).

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Interpretation The potential of the FAST score as a non-invasive monitoring tool to identify histological changes following treatment requires further evaluation and validation.

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Research in context

Evidence before this study

Based on published articles identified on PubMed from Jan 1, 2017, to Dec 31, 2022, people who have non-alcoholic steatohepatitis (NASH) with a disease activity score ≥4 and significant fibrosis (stage ≥2) are at higher risk of progression to cirrhosis (at-risk NASH). No pharmacotherapeutic treatments are currently approved for at-risk NASH, but several candidate drugs are undergoing clinical trials; participation in such trials requires patients to undergo baseline and end-of-treatment biopsies to meet the regulator-approved endpoints of NASH resolution and/or fibrosis improvement. However, biopsies have limitations and are also not practical when considering the future translation of any successful treatments to clinical practice. Therefore, non-invasive methods of assessing treatment effect are needed. The FibroScan-aspartate aminotransferase (FAST) score has been validated for the identification of patients with at-risk NASH and could also be a potential non-invasive measure of treatment effect.

Added value of this study

In this post-hoc analysis of the results of a phase 2b trial of semaglutide 0.1, 0.2, or 0.4 mg once daily *vs* placebo in patients with biopsy-confirmed, non-cirrhotic NASH, the association between changes in FAST score from baseline to week 72 and histological assessment of NASH and fibrosis was examined. The FAST score and its components were reduced in patients who received semaglutide *vs* placebo, and reduction was greater in semaglutide-treated patients who had histological improvement than those who did not. Reduction in FAST score was associated with achievement of the primary endpoint (NASH resolution without worsening in fibrosis) and a reduction in the Non-alcoholic fatty liver disease Activity Score, but not improvement in fibrosis.

Implications of all the available evidence

The FAST score is already validated for the identification of patients with NASH at risk of progression to cirrhosis who are candidates for pharmacotherapy. However, broadening the clinical utility of the FAST score to assess histologic response to treatment requires further investigation and validation in ongoing and future trials.

Introduction

Non-alcoholic steatohepatitis (NASH) is the progressive form of non-alcoholic fatty liver disease (NAFLD), characterised by hepatocyte damage (ballooning) and lobular inflammation.[1] NASH can lead to progressive fibrosis, cirrhosis, and liver decompensation, and as such represents a major unmet medical need.[2] Patients with active steatohepatitis (defined histologically by a NAFLD Activity Score [NAS] ≥4) and significant fibrosis (F ≥2), also known as at-risk NASH,[3,4] are at highest risk of progression to cirrhosis and deleterious outcomes, and are therefore candidates for pharmacotherapy.[2]

Many compounds are currently being tested in therapeutic trials that aim to demonstrate histological improvement, defined as resolution of NASH or fibrosis improvement.[5] Therefore, consecutive liver biopsies are required both to select patients for clinical trials and assess treatment efficacy. However, interpretation of liver histology is subject to considerable inter-observer variability,[6,7] and liver biopsy may not be feasible in the large number of patients in need of therapy in clinical practice. Thus, future access to successful candidate drugs may be severely restricted. Moreover, repeating a liver biopsy to assess treatment effect will not be acceptable to many patients. There is therefore a pressing need to develop non-invasive tests and scores that can both identify patients with at-risk NASH and assess treatment response.[8]

In patients with NAFLD/NASH, FibroScan liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) are non-invasive methods that support the diagnosis of liver fibrosis and steatosis, respectively.[9] Combining FibroScan LSM and CAP with the measurement of aspartate aminotransferase (AST)—known as FibroScan-AST (FAST)—allows the identification of patients with at-risk NASH,[8] as shown in several cohorts encompassing diverse geographical backgrounds and disease severities.[4] In cross-sectional analyses, patients with a FAST score ≥0.67 had a high likelihood of having at-risk NASH, whereas patients

with a score of 0.35 to <0.67 had a moderate likelihood, and those with a score <0.35 had a low likelihood.[4] Using these cut-offs, a meta-analysis of 12 observational studies, with a total of 5835 participants with biopsy-confirmed NAFLD, reported a pooled FAST score sensitivity of 89% (95% CI 82%, 93%) and a pooled specificity of 89% (95% CI 83%, 94%).[10] Although the FAST score appears to have good accuracy for identifying patients in need of pharmacotherapy, its ability to identify histological changes is unknown. If some of the current compounds in development are approved, the pharmacotherapeutic management of people with NASH will be greatly simplified if a single, widely available and easily repeatable score can be used for both patient selection and assessment of treatment response.

Subcutaneous treatment with the glucagon-like peptide-1 analogue semaglutide improves glycaemic control and reduces bodyweight in patients with type 2 diabetes,[11] and assists with weight loss in people with overweight/obesity.[12] In a phase 2b trial in patients with NASH (NCT02970942), semaglutide 0.4 mg once daily led to a significant increase in the proportion of patients achieving resolution of NASH without worsening of fibrosis compared with placebo.[13] Semaglutide is currently being tested in a large, randomised phase 3 trial for the treatment of patients with NASH (ESSENCE, NCT04822181).

In the current study, we performed a post-hoc analysis from the phase 2b trial to evaluate whether changes in FAST score correlate with histological improvement.

Methods

Trial design

Detailed information on the design of the trial has been published previously (Appendix pg 4).[13] Briefly, eligible patients had biopsy-confirmed NASH, F1–3, NAS ≥4, body mass index (BMI) >25 kg/m^2, and glycated haemoglobin ≤10%.[13] Patients were randomised to semaglutide 0.1 mg, 0.2 mg, 0.4 mg, or placebo, given once daily (3:1 ratio of semaglutide:placebo at each dose level); titration started at a dose of 0.05 mg and was escalated in 4-week increments to the randomised dose.[13] Treatment continued for 72 weeks with a follow-up period of 7 weeks. The primary endpoint was NASH resolution with no worsening of liver fibrosis.[13]

Ethics

The original study was approved by an independent ethics committee or institutional review board at each site.[13] Informed consent was obtained from every participant before any trial-related activities took place, including activities to determine suitability for the trial.[13]

FAST measurements

AST, LSM, and CAP (if available) were determined at baseline and at weeks 28, 52, and 72 of treatment. For FibroScan, centres were required to apply quality criteria comprising an interquartile range/median ratio less than 30%, with at least 10 measurements performed. The results of these quality assessments were not available for this analysis and so no data were excluded on this basis. Liver biopsy was performed at baseline and week 72. Other parameters were also recorded at baseline and during treatment.

Post-hoc analyses

The main objective of these post-hoc analyses was to compare changes in FAST score from baseline to week 72 with histological assessment of NASH improvement. This was done using data for the primary endpoint (NASH resolution with no worsening of liver fibrosis), supportive secondary endpoints (improvement of ≥1 stage in liver fibrosis without worsening of NASH and improvement of ≥1 and ≥2 points in NAS), and exploratory endpoints (≥1-point change in steatosis, ballooning, and lobular inflammation scores, and liver fibrosis stage). The association between bodyweight reduction and histological changes was also investigated.

Statistical analysis

Analyses were performed for all randomised patients who had available FAST scores (performed according to the criteria above) and biopsy results at both baseline and end of treatment. No formal sample size calculation was performed. Data are presented for the pooled semaglutide treatment arms and the placebo arm as mean values with standard deviation or standard error of the mean, as appropriate.

Correlations between FAST score and histological changes were analysed using an analysis of covariance. Kendall's tau-b correlations were employed to compare histology with changes in biomarkers. Kruskal–Wallis test p-values were derived to compare FAST scores denoting improvement *vs* no difference and progression within treatment arm. For binary endpoints, the comparison was between achieving and not achieving the endpoint. Receiver operating characteristic (ROC) analyses were performed to assess the associations between changes in FAST score and histology. Further cut-off points generating specific values of sensitivity and specificity were determined by inspection of data tables for Youden index metrics. Statistical analyses were performed using R Version 4.2.

Role of the funding source

The funder was responsible for the study design and contributed to data collection, analysis, and interpretation, and participated in the preparation and review of the manuscript in collaboration with the authors. All authors had full access to the data, and all authors had final responsibility for the decision to submit for publication.

Results

Baseline characteristics

Among 320 patients in the full analysis set, 122 patients (93 who received semaglutide [of whom 32 received 0.1 mg, 32 received 0.2 mg, and 29 received 0.4 mg] and 29 who received placebo) had biopsy and FibroScan results—and therefore FAST scores—at baseline and week 72 and were included for analysis. Of the patients excluded, 11 had no or incomplete biopsy results, six lacked an AST measurement, and 22 had neither biopsy nor AST data. In addition, 118 patients had no LSM/CAP data, 21 lacked both LSM/CAP and biopsy results, and three had biopsy only without LSM/CAP or AST measurements. Seventeen patients lacked all above measures. FibroScan data were available from 88 of the 143 trial centres. There were 25 study centres without the facilities to perform FibroScan measurements and a further 15 that could perform LSM but not CAP. All countries represented had at least some centres with FibroScan capability except for Greece.

The characteristics of the 122 patients with available biopsy and FAST scores are shown in Table 1 (there were no meaningful differences in the baseline characteristics of the 122 patients who were included in the current analysis *vs* 198 patients who were not included; Appendix pg 8). Approximately 80% of patients were aged <65 years, the mean BMI was around 35 kg/m^2, NAS was 4.7, and approximately half of the patients had fibrosis stage 3. Overall, the characteristics of this subpopulation were comparable to the total population reported in the primary analysis of the phase 2b trial.[13] More patients in the pooled semaglutide group compared with the placebo group were female (62% *vs* 45%) and had type 2 diabetes (63% *vs* 55%) (Table 1). Mean baseline FAST scores (standard deviation) were 0.58 (0.22) in the pooled semaglutide group and 0.54 (0.21) in the placebo group. Values for FAST score components are shown in Table 1.

FAST score reduction following semaglutide treatment

There was a significant reduction in FAST score in the pooled semaglutide group *vs* placebo after 72 weeks, with differentiation evident after 28 weeks (Fig. 1). The estimated mean change (±standard error) from baseline in FAST score with placebo was 0.19 (±0.13) at week 72. Placebo-adjusted mean changes in FAST score for semaglutide were –0.13 (±0.06) with the 0.1 mg dose ($p = 0.030$ *vs* placebo), –0.23 (±0.06) with 0.2 mg ($p < 0.001$), and –0.31 (±0.06) with 0.4 mg ($p < 0.001$) (Appendix pg 5). All three components of the FAST score—AST level, CAP, and particularly LSM—showed reductions in the pooled semaglutide group that were not evident or as pronounced in the placebo group (Fig. 1).

Among patients with a FAST score ≥0.67 at baseline (high likelihood of at-risk NASH), 53% of patients in the pooled semaglutide group had a FAST score

	Patients with FAST score and biopsy at baseline and week 72 (N = 122)	
	Semaglutide pooled (N = 93)	Placebo (N = 29)
Age, <65/≥65 years, n (%)	73 (78.5)/20 (21.5)	24 (82.8)/5 (17.2)
Sex		
Female, n (%)	58 (62.4)	13 (44.8)
Male, n (%)	35 (37.6)	16 (55.2)
Type 2 diabetes, n (%)	59 (63.4)	16 (55.2)
Bodyweight (kg), mean (SD)	95.6 (21.2)	101.85 (25.8)
Body mass index (kg/m^2), mean (SD)	35.8 (6.15)	35.2 (6.1)
Liver enzymes (U/L), geometric mean (CV)		
Alanine aminotransferase	54 (0.57)	58 (0.58)
Aspartate aminotransferase	43 (0.51)	42 (0.47)
FibroScan measurements, mean (SD)		
LSM (kPa)	12.51 (7.09)	10.27 (4.35)
CAP (dB/m)	333.84 (55.00)	345.86 (34.27)
FAST score, mean (SD)	0.58 (0.22)	0.54 (0.21)
Fibrosis stage, n (%)		
1	25 (26.9)	7 (24.1)
2	18 (19.4)	6 (20.7)
3	50 (53.8)	16 (55.2)
Total NAS[a], mean (SD)	4.75 (0.9)	4.7 (0.9)

Subset analysis includes all patients with FAST score at baseline and week 72. Semaglutide pooled group includes doses of 0.1, 0.2, and 0.4 mg once daily. CAP, controlled attenuation parameter; CV, coefficient of variation; FAST, FibroScan-aspartate aminotransferase; LSM, liver stiffness measurement; NAS, Non-alcoholic fatty liver disease Activity Score; SD, standard deviation. [a]Range 0–8 (unweighted sum of the scores for steatosis [0–3], lobular inflammation [0–3], and ballooning [0–2]).

Table 1: **Baseline characteristics of the analysis subset.**

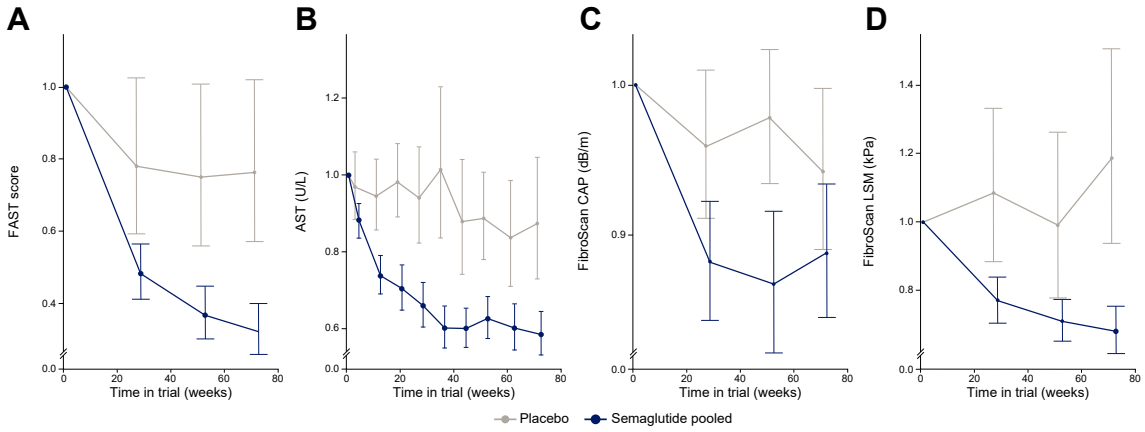

Fig. 1: Ratio to baseline for change in FAST score and components from baseline to week 72, pooled semaglutide treatment and placebo groups: (A) FAST score; (B) AST; (C) FibroScan CAP; (D) FibroScan LSM. Subset analysis includes all patients with FAST score at baseline and week 72. Semaglutide pooled group includes doses of 0.1, 0.2, and 0.4 mg once daily. AST, aspartate aminotransferase; CAP, controlled attenuation parameter; FAST, FibroScan-aspartate aminotransferase; LSM, liver stiffness measurement.

<0.35 (low risk) at week 72 (*vs* 30% in the placebo-treated group). Among patients with a FAST score 0.35 to <0.67 (indeterminate risk) at baseline, 78% of patients in the pooled semaglutide group had a FAST score <0.35 at week 72 (*vs* 27% in the placebo-treated group) (Table 2).

Association between reduction in FAST score and improvement in histological endpoints

Reduction in FAST score from baseline to week 72 was greater in patients who met histological endpoints than in those who did not (Table 3). In the pooled semaglutide group, reductions in FAST score at week 72 were greater in patients who achieved the primary endpoint *vs* those who did not (–0.40 points *vs* –0.22 points; p = 0.002) (Table 3) indicating that reduction in FAST score was associated with achievement of NASH resolution without worsening in fibrosis. Reductions were generally numerically greater in the semaglutide pooled group *vs* the placebo group; however, in the placebo group, reductions in FAST score at week 72 were also greater in patients who achieved the primary endpoint *vs* those who did not (–0.25 *vs* 0.00; p = 0.047).

Regardless of semaglutide or placebo treatment, reduction in FAST score at week 72 was associated with improvement in NAS, assessed by histology (Fig. 2, Table 3). In both groups, reductions in FAST score at week 72 were greater in patients with improvement in NAS ≥1 *vs* those without (semaglutide pooled group: –0.36 *vs* –0.08; p < 0.001; placebo group: –0.25 *vs* 0.06; p = 0.001) and in those with improvement in NAS ≥2 *vs* those without (semaglutide pooled group: –0.40 *vs* –0.14; p < 0.001; placebo group: –0.29 *vs* 0.051; p < 0.001), assessed by histology (Fig. 2, Table 3). In both groups, a decrease in FAST score was not associated with fibrosis improvement with no worsening in NASH (Table 3).

Analysis of exploratory endpoints suggested that, regardless of treatment with semaglutide or placebo, the reduction in FAST score from baseline to week 72 was greater in patients who had improvement in steatosis or ballooning (*vs* those who did not) but was not associated with improvement in fibrosis (Table 3). At week 72,

FAST score (baseline)	Treatment group	FAST score (week 72) (N = 122)		
		<0.35, % (n)	0.35 to <0.67, % (n)	≥0.67, % (n)
≥0.67	Semaglutide pooled	52.6 (20)	23.7 (9)	23.7 (9)
	Placebo	30.0 (3)	20.0 (2)	50.0 (5)
0.35 to <0.67	Semaglutide pooled	77.5 (31)	20.0 (8)	2.5 (1)
	Placebo	27.3 (3)	36.4 (4)	36.4 (4)
<0.35	Semaglutide pooled	86.7 (13)	13.3 (2)	0 (0)
	Placebo	75.0 (6)	12.5 (1)	12.5 (1)

Subset analysis includes all patients with FAST score at baseline and week 72. Semaglutide pooled group includes doses of 0.1, 0.2, and 0.4 mg once daily. FAST, FibroScan-aspartate aminotransferase; NASH, non-alcoholic steatohepatitis.

Table 2: **Proportion of patients at risk of NASH progression according to FAST cut-offs with semaglutide *vs* placebo.**

Histological response	Treatment	Endpoint met?	Week 28			Week 72		
			N	Mean (95% CI)	p-value	N	Mean (95% CI)	p-value
NASH resolution with no worsening of fibrosis (primary endpoint)	Semaglutide pooled	No	43	−0.212 (−0.662, 0.237)	0.12	43	−0.219 (−0.735, 0.297)	**0.002**
		Yes	45	−0.272 (−0.712, 0.168)		46	−0.398 (−0.840, 0.043)	
	Placebo	No	21	0.008 (−0.438, 0.455)	0.15	21	0.003 (−0.492, 0.497)	**0.047**
		Yes	7	−0.143 (−0.636, 0.349)		7	−0.245 (−0.720, 0.231)	
Improvement in liver fibrosis and no worsening of NASH (confirmatory secondary endpoint)	Semaglutide pooled	No	49	−0.254 (−0.741, 0.232)	0.44	49	−0.313 (−0.855, 0.230)	0.92
		Yes	39	−0.228 (−0.621, 0.165)		40	−0.310 (−0.778, 0.158)	
	Placebo	No	20	0.022 (−0.450, 0.495)	0.12	20	−0.018 (−0.563, 0.526)	0.39
		Yes	8	−0.159 (−0.526, 0.208)		8	−0.161 (−0.609, 0.288)	
Improvement in NAS ≥1	Semaglutide pooled	No	15	−0.078 (−0.434, 0.278)	**0.001**	15	−0.077 (−0.530, 0.376)	**<0.001**
		Yes	77	−0.275 (−0.701, 0.152)		78	−0.356 (−0.823, 0.112)	
	Placebo	No	17	0.087 (−0.356, 0.531)	**<0.001**	17	0.064 (−0.402, 0.529)	**0.001**
		Yes	11	−0.210 (−0.433, 0.014)		11	−0.249 (−0.637, 0.139)	
Improvement in NAS ≥2	Semaglutide pooled	No	32	−0.151 (−0.524, 0.223)	**0.002**	32	−0.143 (−0.564, 0.278)	**<0.001**
		Yes	60	−0.291 (−0.734, 0.151)		61	−0.399 (−0.859, 0.062)	
	Placebo	No	19	0.063 (−0.379, 0.505)	**<0.001**	19	0.051 (−0.393, 0.496)	**<0.001**
		Yes	9	−0.225 (−0.463, 0.013)		9	−0.292 (−0.671, 0.086)	
Improvement in hepatocyte ballooning	Semaglutide pooled	No	25	−0.151 (−0.583, 0.281)	**0.011**	25	−0.146 (−0.651, 0.359)	**<0.001**
		Yes	67	−0.277 (−0.701, 0.148)		68	−0.371 (−0.824, 0.082)	
	Placebo	No	13	0.085 (−0.403, 0.572)	**0.02**	13	0.085 (−0.434, 0.604)	**0.012**
		Yes	15	−0.128 (−0.491, 0.235)		15	−0.184 (−0.591, 0.223)	
Improvement in steatosis	Semaglutide pooled	No	33	−0.141 (−0.493, 0.211)	**<0.001**	34	−0.199 (−0.666, 0.267)	**<0.001**
		Yes	59	−0.299 (−0.744, 0.146)		59	−0.375 (−0.861, 0.112)	
	Placebo	No	20	0.031 (−0.450, 0.512)	**0.025**	20	0.053 (−0.356, 0.462)	**<0.001**
		Yes	8	−0.181 (−0.454, 0.092)		8	−0.339 (−0.702, 0.024)	
Improvement in lobular inflammation	Semaglutide pooled	No	51	−0.227 (−0.670, 0.217)	0.55	52	−0.276 (−0.732, 0.180)	0.059
		Yes	41	−0.262 (−0.696, 0.171)		41	−0.355 (−0.910, 0.199)	
	Placebo	No	21	0.049 (−0.389, 0.488)	**0.002**	21	0.019 (−0.506, 0.543)	**0.008**
		Yes	8	−0.226 (−0.464, 0.012)		8	−0.232 (−0.572, 0.107)	
Improvement in fibrosis	Semaglutide pooled	No	48	−0.253 (−0.745, 0.238)	0.48	48	−0.322 (−0.854, 0.210)	0.66
		Yes	40	−0.230 (−0.618, 0.158)		41	−0.299 (−0.782, 0.184)	
	Placebo	No	19	0.013 (−0.465, 0.491)	0.29	19	−0.031 (−0.578, 0.516)	0.68
		Yes	9	−0.119 (−0.536, 0.298)		9	−0.118 (−0.608, 0.373)	

Subset analysis includes all patients with FAST score at baseline and week 72. Semaglutide pooled group includes doses of 0.1, 0.2, and 0.4 mg once daily. Significant p-values are bolded. CI, confidence interval; FAST, FibroScan-aspartate aminotransferase; NAS, Non-alcoholic fatty liver disease Activity Score; NASH, non-alcoholic steatohepatitis.

Table 3: **Change in FAST score (baseline to week 28 and week 72) and association with histological response at week 72.**

reduction in FAST score was also greater in patients with improvement in lobular inflammation, but only reached statistical significance in the placebo group. A reduction in FAST score at week 28 was associated with improvement in NAS ≥1 or ≥2, ballooning, and steatosis in both groups, and was also associated with improvement in lobular inflammation in the placebo group. However, the reduction in FAST score at week 28 was not predictive of achievement of the primary endpoint (Table 3).

Reduction in individual FAST components and bodyweight and association with NASH resolution with no worsening in fibrosis

Analysis of the individual components of FAST score and their association with the primary endpoint illustrated that regardless of treatment (semaglutide or placebo), reductions in AST, CAP, and LSM from baseline to week 72 were not associated with meeting the primary endpoint (Fig. 3, Appendix pg 6). In the pooled semaglutide group, AST decreased by 21.9 U/L *vs* 17.0 U/L in patients who met the primary endpoint *vs* those who did not (p = 0.12); CAP decreased by 45.8 dBm *vs* 20.0 dBm, respectively (p = 0.051), and LSM decreased by 4.9 kPa *vs* 3.6 kPa, respectively (p = 0.34). In the placebo group, reductions in the individual FAST components were also not associated with meeting the primary endpoint (Fig. 3, Appendix pg 6).

In the pooled semaglutide group, reduction in bodyweight was significantly greater in patients who met the primary endpoint *vs* those who did not (weight change −11.5 kg *vs* −5.1 kg; p < 0.001). The association

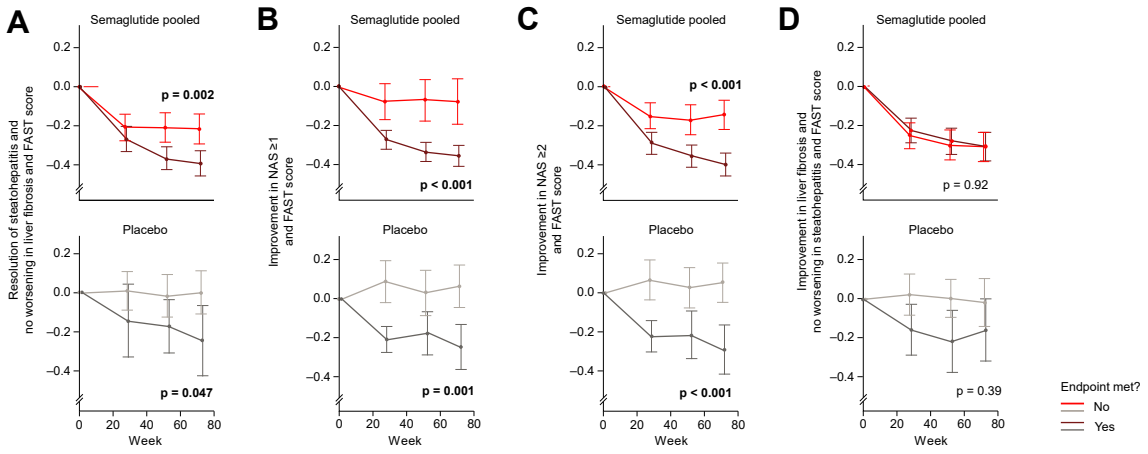

Fig. 2: Association between reduction in FAST score and histological response: (A) NASH resolution and no worsening in fibrosis; (B) Improvement in NAS ≥2; (C) Improvement in NAS ≥1; (D) Improvement in fibrosis and no worsening in NASH. Subset analysis includes all patients with FAST score at baseline and week 72. Semaglutide pooled group includes doses of 0.1, 0.2, and 0.4 mg once daily. p-values are for the difference between groups at week 72. FAST, FibroScan-aspartate aminotransferase; NAS, Non-alcoholic fatty liver disease Activity Score; NASH, non-alcoholic steatohepatitis.

was not statistically significant in the placebo group (Fig. 3). The association between bodyweight change and achievement of additional histological endpoints is shown in Appendix pg 2.

FAST score reduction associated with NASH resolution without worsening in fibrosis in the pooled semaglutide group

Reduction in FAST score was associated with NASH resolution without worsening in fibrosis in the pooled semaglutide group with an area under the ROC curve (AUC) of 0.69 (95% CI 0.58, 0.81) (Fig. 4). AUC and p-values for comparison of FAST score with its individual components were 0.60 (p = 0.045), 0.62 (p = 0.191), and 0.56 (p = 0.013) for AST, CAP, and LSM, respectively. Using the Youden index in the ROC curve analysis, a reduction of the FAST score of 0.22 points after semaglutide treatment was associated with achievement of the primary endpoint, with a sensitivity of 78% and a specificity of 60% (Table 4). The positive and negative likelihood ratios and odds ratio were 1.26, 0.25, and 4.93, respectively. In the placebo group, a reduction in FAST score of 0.22 points had a sensitivity of 57% and a specificity of 81% for association with the primary endpoint. The positive and negative likelihood ratios and odds ratio were 1.38, 0.38, and 3.69, respectively.

Discussion

Currently, improvement in liver histology is required to evaluate the efficacy of experimental pharmacotherapies for the treatment of NASH in pivotal studies.[14,15] Given the invasive nature of liver biopsy, a validated, non-invasive method of efficacy assessment that could also be applied clinically would represent a major advancement.

We previously demonstrated that semaglutide treatment reduced AST, alanine aminotransferase (ALT), LSM, and CAP compared with placebo in patients with NASH.[13] Apart from ALT, the other parameters above are included in the FAST score, which was developed primarily to identify patients with NASH at risk of progressive disease.[4] In the present study, we investigated: a) whether the FAST score was reduced following semaglutide treatment and b) if the FAST score could be used to identify histological improvement.

FAST score was reduced to a greater extent after treatment with semaglutide than with placebo. More patients with a FAST score ≥0.67 at baseline had a score <0.35 at the end of treatment with semaglutide *vs* placebo (53% *vs* 30%). Furthermore, investigation of the association between reduction in score and histological endpoints found that reductions in FAST score were more pronounced in patients who had a positive outcome for the primary biopsy-based endpoint, ie, NASH resolution with no worsening in fibrosis, as well as improvement in NAS of ≥1 or ≥2, compared with those who did not. For semaglutide-treated patients, a reduction in FAST score of at least 0.22 points was associated with a positive outcome for the primary endpoint. The negative likelihood ratio suggests that the FAST score may also be useful for identifying patients who are less likely to have histological improvements with semaglutide (change in score less than −0.22 points). A recent analysis of the performance of various non-invasive scores in assessing the treatment effect of obeticholic acid in the phase 3

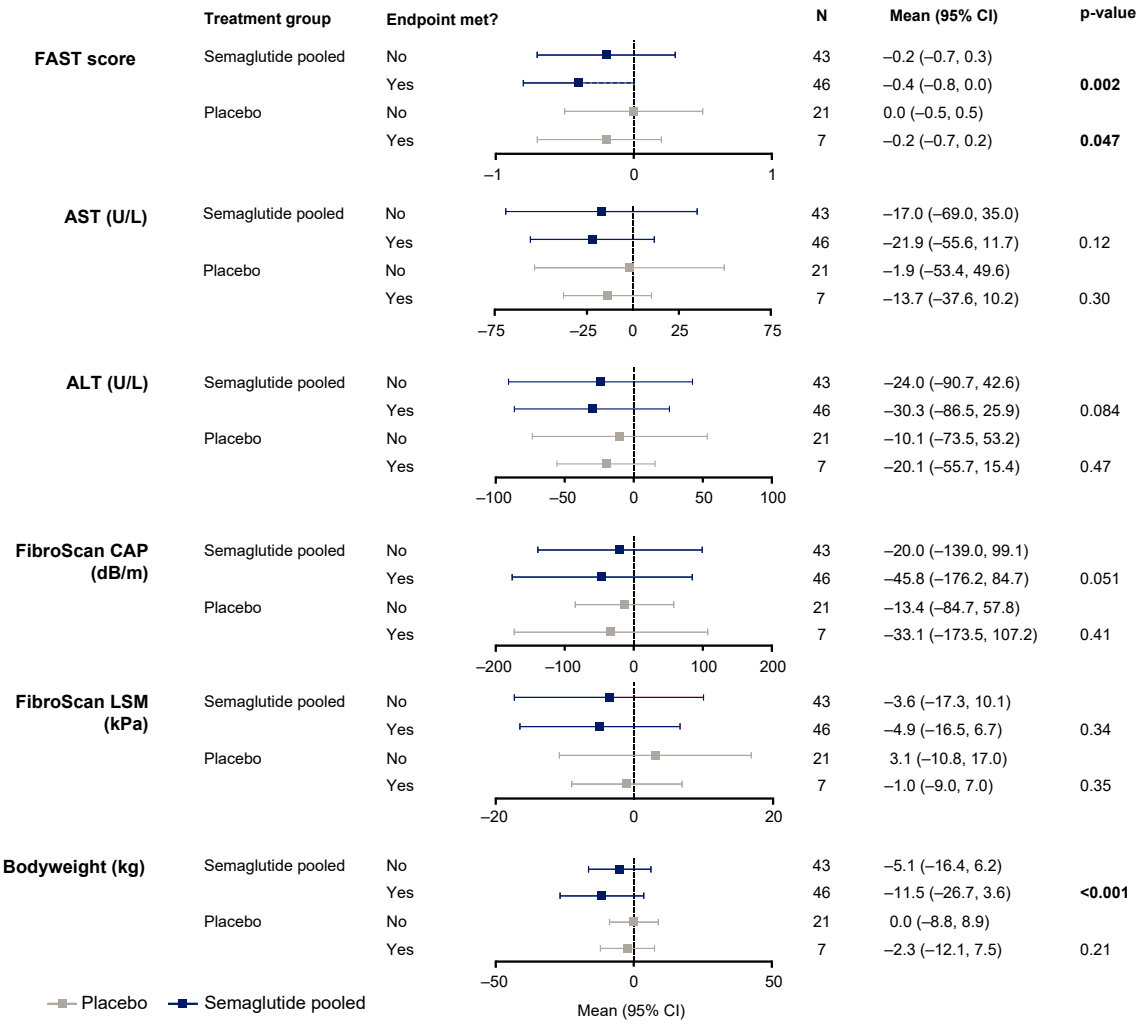

Fig. 3: Changes in FAST score and its components, and changes in bodyweight, in relation to NASH resolution without worsening in fibrosis at week 72. Subset analysis includes all patients with FAST score at baseline and week 72. Semaglutide pooled group includes doses of 0.1, 0.2, and 0.4 mg once daily. p-values are from Kruskal–Wallis test. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CI, confidence interval; FAST, FibroScan-aspartate aminotransferase; LSM, liver stiffness measurement; NASH, non-alcoholic steatohepatitis.

REGENERATE study also indicated that selected scores could distinguish treatment responders from non-responders.[16] However, that study focussed on fibrosis improvement and not NASH resolution, in agreement with the positive outcome of the interim 72-week analysis of that study.[17]

Reductions in FAST score at week 72 were associated with NASH resolution as well as improvement in NAS of ≥1 or ≥2 points, regardless of semaglutide or placebo treatment, although FAST score reductions seemed more pronounced with semaglutide than with placebo in patients who met the histological endpoints (Appendix pg 6). This may be in part explained by patients in the semaglutide pooled group generally exhibiting greater reductions in CAP, and to a lesser extent AST and LSM, than those receiving placebo. In placebo-treated patients, a reduction in the FAST score was also associated with a positive outcome for the primary biopsy-based endpoint, ie, NASH resolution with no worsening in fibrosis, as well as improvement in NAS of ≥1 or ≥2 points. The FAST cut-off of a 0.22-point reduction was identified in the pooled semaglutide group (doses of 0.1–0.4 mg once daily). With placebo, a reduction in FAST score of at least 0.22 points had an even higher specificity for a positive outcome, but a lower sensitivity, compared with the pooled semaglutide group. Further studies will be needed to determine the potential use of FAST score reduction as a surrogate endpoint.

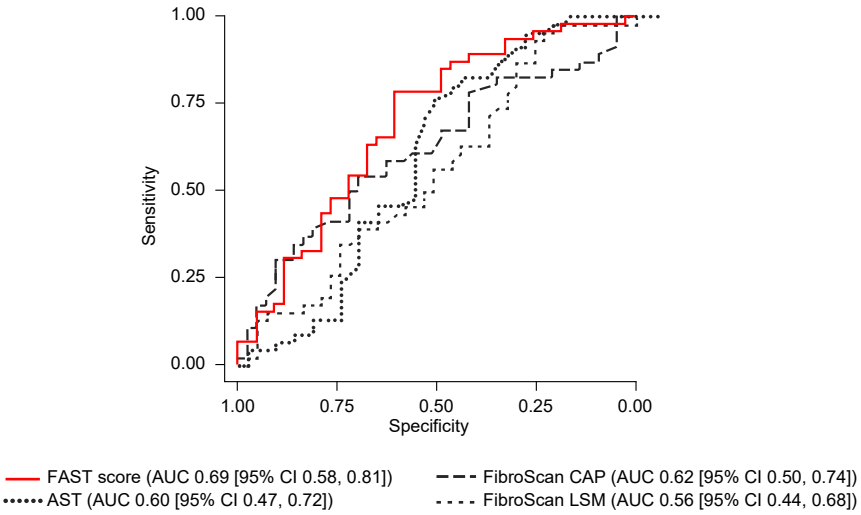

Fig. 4: Change in FAST score and components (AST, FibroScan CAP, FibroScan LSM) from baseline to week 72 and association with NASH resolution without worsening of fibrosis—receiver operating characteristic curve for patients in the semaglutide treatment pool with FAST score who met the primary endpoint at week 72 (on treatment). Subset analysis includes all patients with FAST score at baseline and week 72. Semaglutide pooled group includes doses of 0.1, 0.2, and 0.4 mg once daily. AST, aspartate aminotransferase; AUC, area under the curve; CAP, controlled attenuation parameter; CI, confidence interval; FAST, FibroScan-aspartate aminotransferase; LSM, liver stiffness measurement; NASH, non-alcoholic steatohepatitis.

The association between a reduction in FAST score and improvement in NAS of ≥1 or ≥2 points was observed at both 28 weeks and 72 weeks. Reductions in FAST score were also associated with improvements in steatosis and ballooning at both time points. These observations suggest the FAST score may predict improvement in NAS and ballooning at earlier time points. In patients treated with placebo, reduction in FAST score was also associated with improvement in lobular inflammation at both 28 weeks and 72 weeks. A similar trend was observed in patients treated with semaglutide at week 72, although it did not reach statistical significance.

Four patients who met the primary endpoint had an increase in FAST score. In three of these patients, the increase was ≤0.06 and thus could be considered related to measurement variability, particularly for the elastography component of the FAST score.[18] The other patient had an increase of >0.22 in FAST score, driven by a profound increase (>10 kPa) in LSM from week 52 to 72 but without significant change in AST or CAP. Since this patient had an improvement in liver fibrosis, the large LSM increase could be due to an incorrect assessment.

Of interest, no increase in FAST score was observed in patients who did not meet the primary endpoint, nor in those who had stable disease or progression in histological disease activity. It should be noted that very few patients receiving semaglutide had a worsening in NAS score (6/93 in the pooled semaglutide arms; 7/28 in the placebo group). It is possible that the time frame of the study was not sufficient to capture progression driven by natural disease course. Thus, from these observations, we are unable to conclude if FAST score can be used as a biomarker for monitoring disease progression. Further studies of longer duration may demonstrate if FAST score reduction can be used to predict clinical outcomes resulting from a treatment effect. The phase 3 ESSENCE trial of semaglutide is currently ongoing and will be used to evaluate the present findings in both a

	Sensitivity ≥90%	Specificity ≥90%	Youden index
Threshold[a]	−0.06	−0.58	−0.22
Sensitivity/specificity	0.91/0.33	0.17/0.91	0.78/0.60
n positive/total N	71/89	12/89	53/89

Thresholds were determined based on receiver operating characteristic curve analysis. Subset analysis includes all patients with FAST score at baseline and week 72. Semaglutide pooled group includes doses of 0.1, 0.2, and 0.4 mg once daily. FAST, FibroScan-aspartate aminotransferase; NASH, non-alcoholic steatohepatitis. [a]Denotes change from baseline.

Table 4: **Reduction in FAST score of 0.22 points and association with achievement of the primary endpoint (resolution of NASH with no worsening of fibrosis) in the pooled semaglutide treatment group.**

larger and independent cohort, and will also include clinical outcome data when available.

In contrast to the association with NASH resolution, no association between reduction in FAST score and improvement in fibrosis alone was shown. There are several hypothetical explanations for this dissociation of response. One possibility is that the kinetics of steatohepatitis improvement/resolution, and those of fibrosis improvement, differ markedly, ie, the time frame for fibrosis reduction is much longer than for reduction in disease activity. This has been shown even upon elimination of the cause of the liver disease (eg, eradication of viral hepatitis[19]) and is even more relevant for NASH where the metabolic dysfunction is improved, but not cured, by current therapies. Another possibility is that marked weight loss and improvement in necroinflammation were the main drivers of the reduction in all three components of the FAST score, and this might mask the correlation with fibrosis improvement. An alternative explanation is that the FAST score may be more sensitive to changes in disease activity (the steatohepatitis component of the at-risk NASH histological composite) than to changes in fibrosis. Thus, reduction in FAST score and associated LSM values may not necessarily reflect improvement of liver fibrosis. Indeed, the substantial weight loss induced by semaglutide was associated with improvement in steatosis and lobular inflammation (Appendix pg 6). It is therefore conceivable that, although FAST was initially validated as a composite marker of a composite histological feature (active steatohepatitis with significant fibrosis), it is impacted to a larger degree by steatosis and/or steatohepatitis, and changes therein, than by fibrosis and fibrosis changes.

It is of interest that bodyweight reduction was associated with an improvement in histological endpoints in the pooled semaglutide group, since using bodyweight changes to identify improvement in NASH would be simple, inexpensive, and convenient. For both FAST score reduction and for bodyweight reduction, we found a clear association to the primary endpoint. However, bodyweight reduction with concomitant improvements in liver histology have only been reported for semaglutide and liraglutide.[13,20] As a result, change in bodyweight is unlikely to find widespread clinical utility as a monitoring tool for improvements in histological endpoints. In contrast, the FAST score may be applicable as a general marker of NASH resolution for other pharmacotherapies.

This study has several limitations. The elastography measurements were performed locally according to standardised guidance regarding quality and reproducibility, but information on quality measurements of the scans and probe type were lacking. Elastography is operator-dependent and LSMs can have significant variability unrelated to fibrosis changes.[18] Furthermore, the current post-hoc analysis represents only a subcohort of the phase 2b trial because FibroScan data were not available from all centres. Despite this, there was a low probability of selection bias since this subpopulation was largely representative of the main cohort.[13] Due to relatively low patient numbers, data from all three semaglutide treatment arms were pooled to maximise the power to detect any associations between FAST score and histological improvement. Recently, a multi-society consensus statement has introduced non-stigmatising nomenclature for "fatty" liver diseases.[21] Metabolic dysfunction-associated steatotic liver disease (MASLD) is the replacement term for NAFLD, with metabolic dysfunction-associated steatohepatitis (MASH) replacing NASH.[21] Due to the time period, these new terms were unable to be used in the current study. However, the patient population and resulting data presented herein would likely best apply to MASLD given its definition as the presence of hepatic steatosis in the presence of ≥1 metabolic risk factor.[21] Finally, we acknowledge that the histological reference standard against which FAST performance is measured is subject to inter- and intra-observer variation.

In conclusion, in this post-hoc analysis we showed that an improvement in the FAST score was associated with NASH resolution with no worsening of fibrosis at week 72 of treatment with semaglutide. In the current setting, our findings indicate a significant, but modest, performance of the FAST score in meeting the primary endpoint (sensitivity 78%; specificity 60%; AUC 0.69). Therefore, the FAST score as a practical tool for clinicians to assess histologic response to semaglutide in patients with NASH requires further evaluation and independent validation.

Contributors

LMN, MSK, SL, and VN were responsible for the study concept and design, and data synthesis and analysis. VWSW, QMA, AG, JG, PNN, and VR recruited patients and collected data as investigators in the original trial. All authors had access to the data and contributed equally to drafting and critically revising the manuscript. SL and VR accessed and verified the data.

Data sharing statement

Data will be shared with bona fide researchers who submit a research proposal approved by the independent review board. Individual participant data will be shared in data sets in a de-identified and anonymised format. Data will be made available after research completion and approval of the product and product use in the European Union and the USA. Information about data access request proposals can be found at novonordisk-trials.com.

Declaration of interests

VWSW served as a consultant or advisory board member for AbbVie, Boehringer Ingelheim, Echosens, Gilead Sciences, Intercept, Inventiva, Novo Nordisk, Pfizer, Sagimet Biosciences, and TARGET PharmaSolutions; and a speaker for Abbott, AbbVie, Gilead Sciences, Novo Nordisk, and Unilab. He has received a research grant from Gilead Sciences, and is a co-founder of Illuminatio Medical Technology Limited. He has received travel support from AbbVie and Gilead Sciences.

QMA is supported by the NIHR Newcastle Biomedical Research Centre and the Innovative Medicines Initiative (IMI2) programme of the European Union. Has received consulting fees on behalf of Newcastle University from Alimentiv, Akero, AstraZeneca, Axcella, 89bio, Boehringer Ingelheim, Bristol Myers Squibb, Galmed, Genfit, Genentech, Gilead, GlaxoSmithKline, Hanmi, HistoIndex, Intercept, Inventiva,

Ionis, IQVIA, Janssen, Madrigal, Medpace, Merck, NGMBio, Novartis, Novo Nordisk, PathAI, Pfizer, Pharmanest, Prosciento, Poxel, Resolution Therapeutics, Roche, Ridgeline Therapeutics, RTI, Shionogi, and Terns. He has received payment or honoraria from Fishawack, Integritas Communications, Kenes, Novo Nordisk, Madrigal, Medscape, and Springer Healthcare.

AG and JG declare no conflict of interests.

LMN, MSK, VN and SL are full-time employees and/or shareholders in Novo Nordisk A/S.

PNN was supported by the NIHR Birmingham Biomedical Research Centre. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. He has received consulting fees from Novo Nordisk, Boehringer Ingelheim, Gilead, Intercept, Poxel Pharmaceuticals, BMS, Pfizer, Sun Pharma, Madrigal, and GSK; grants/contracts from Novo Nordisk; and payment or honoraria from Novo Nordisk and AiCME. He has received travel support from Novo Nordisk.

VR has received consulting fees from Novo Nordisk, Terns, Madrigal, Enyo, Poxel, Bristol Myers Squibb, Intercept, NorthSea, and Sagimet. He has received research grants from Gilead Sciences and Intercept Pharmaceuticals, and travel support from Gilead Sciences.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102310.

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