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ORIGINAL ARTICLE

Noninvasive measure of treatment response in non-alcoholic steatohepatitis: Insights from EMMINENCE and meta-analysis

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clinical trials methodology, liver histology, non-alcoholic steatohepatitis, serologic test.

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Abstract

Background and Aim: Liver histology changes are the current gold standard for evaluating non-alcoholic steatohepatitis (NASH), but are limited by their invasiveness and variability for sampling and interpretation. We evaluated noninvasive biomarkers as an indication of histologic changes in NASH.

Methods: Associations between 12-month biomarker and NASH Clinical Research Network histologic score changes in 339 patients with NASH in the EMMINENCE trial was examined with multivariable models and partial canonical correlation. A meta-analysis of 17 NASH trials including 3717 patients examined associations between these same changes and histologic response within treatment groups, and treatment effects on biomarkers and on liver histology. Biopsy measures assessed were changes in ballooning, steatosis, inflammation, and fibrosis, NASH improvement without worsening of fibrosis, and fibrosis improvement without worsening of NASH. All analytic methods suggest that a combination of aspartate aminotransferase (AST), cytokeratin-18 (CK-18 [M30 or M65]), and hemoglobin A1C (HbA1c) changes best predicts overall liver biopsy changes in response to interventions.

Results: The weighted average of standardized mean changes ($0.403 \times$ AST, $0.314 \times$ CK-18, $0.283 \times$ HbA1c) facilitated comparisons of within-group responses and treatment effects among studies included in the meta-analysis. This composite in EMMINENCE discriminated between patients with and without NASH resolution without worsening fibrosis with area under the receiver-operator characteristic curve of 0.7880, and for fibrosis improvement without NASH worsening of 0.7553.

Conclusion: A composite score based on changes in AST, HbA1c, and CK-18 could serve as a surrogate for liver histologic improvement and an effective objective, non-invasive tool for comparative assessment of treatment effects of novel interventions.

Introduction

Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are a global pandemic and their incidence is rapidly increasing.[1,2] NASH can progress to hepatocellular carcinoma or cirrhosis, and is the second most common reason for liver transplantation in the United States.[3,4] As of yet, no drug or intervention has been shown to be effective in improving NASH and hence multiple studies have been commenced. NASH is diagnosed on liver biopsy as hepatic steatosis, lobular inflammation, and ballooning, with or without fibrosis.[5,6] Both the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) require biopsies for the diagnosis of NASH and more importantly require changes in liver related parameters to establish effectiveness of novel interventions for NASH.[7,8] Therefore, in virtually all studies examining new interventions in NASH, "paired" liver biopsies, that is, a biopsy before and a biopsy after 6–18 months of treatment, are being performed in order to determine the effectiveness of new interventions.[9–25] However, liver biopsies are a significant hurdle in NASH studies. First, they are prone to large sampling error[26] and are limited by significant intra- and inter-observer variability.[27] Second, and most importantly, they represent significant patient burden invasiveness, and the associated risks of morbidity[28] and potentially even mortality. This risk, especially in the context of a clinical study, leads to significant limitations in conducting larger NASH studies.

The EMMINENCE study was a phase 2 dose-ranging study of MSDC-0602K, a second-generation insulin sensitizer,

designed to assess effects on liver histology.[21] While some treatment effects on secondary histological endpoints including 12-month change in NAFLD activity score (NAS) and steatosis were observed, the effects on the primary histologic outcome—2-point NAS improvement without worsening fibrosis—were not statistically significant. Although 20.3, 20.2, 28.0, and 31.4% of patients in the placebo and MSDC-0602K 62.5, 125, and 250 mg groups demonstrated NASH improvement without worsening fibrosis and 21.6, 23.8, 28.0, and 29.1% demonstrated fibrosis improvement without worsening NASH, the results were not statistically significant. MSDC-0602K was found to have significant effects on insulin sensitivity and liver injury markers. In a post hoc analysis of the EMMINENCE study, we observed that liver biopsy interpretation and especially the interpretation of accepted trial endpoints of improvement in NASH without worsening of fibrosis or improvement in fibrosis without worsening of NASH were fraught with significant inter-reader variability and hence are highly unreliable measures of treatment effect, substantially reducing our ability to detect a beneficial effect in NASH.[27]

In the present analysis, we sought to explore, both in the EMMINENCE study and in a meta-analysis of clinical trials that examined the effects of NASH interventions using paired liver biopsies, noninvasive measures of histologic improvement that could potentially replace liver biopsies and their interpretation in NASH research.

Methods

EMMINENCE was a phase 2 study examining the effects of three doses of MSDC-0602K *versus* placebo on liver histology in 392 patients with biopsy-confirmed NASH.[21] Patients provided written informed consent, and the protocol and consent form were approved by applicable institutional review boards. Baseline and 12-month biopsies were scored by a single expert hepatopathologist using the NASH Clinical Research Network (CRN) scoring criteria.[29] Fasting blood samples for routine clinical chemistry as well as frozen samples for biomarker assays were analyzed centrally. In this post hoc analysis, multivariable models and partial canonical correlation were used to examine the associations between changes in biomarkers and changes in histological scores from baseline to 12 months in the 339 patients with paired biopsies. The trial was registered on ClinicalTrials.gov (NCT02784444).

Meta-regressions of NASH clinical trials were performed to examine associations between biomarker and histological changes within treatment groups, and to additionally examine associations between treatment effects on biomarkers and on liver histology. Trials that met the following criteria were included:

- Randomized controlled clinical trial
- Paired biopsies performed at baseline and follow-up, scored using the NASH CRN system.
- NASH diagnosis
- Published results at baseline, follow-up, or changes from baseline for any of the following liver injury markers or biomarkers: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, hemoglobin A1c (HbA1c), gamma-glutamyltransferase (GGT), insulin, cytokeratin-18 (CK-18), or enhanced liver fibrosis (ELF) score. Variables selected were based on variables whose change was commonly reported in multiple studies.
- Changes in histological scores for ballooning, inflammation, steatosis, and fibrosis, or the predetermined endpoints of NASH resolution without worsening fibrosis or improvement in fibrosis with no worsening of NASH.
- Sample size of at least 20 subjects in each arm of the study.

All results from a search of PubMed for the keywords ("nonalcoholic steatohepatitis" or "non-alcoholic steatohepatitis") and ("clinical trial" or "randomized") and filtered for "Clinical Trial" were reviewed. EudraCT and ClinicalTrials.gov were searched for the keyword "nonalcoholic steatohepatitis" with results further filtered for phase 2 or 3, interventional, terminated, or completed trials. These three sources returned 265 (PubMed), 153 (ClinicalTrials.gov), and 56 (EudraCT) results, which were each individually reviewed for inclusion in the analysis. Data extracted by one analyst from each source that included means and SDs at baseline, end of study, and changes from baseline to end of study for continuous variables were verified and corrected by a second analyst. Where SDs were not available, any provided estimate of variation was used to derive the SD where possible; a plot-digitizer program was used where data were presented in figures only. Data for dichotomous endpoints were extracted as the number of subjects with the event and corresponding sample size.

All studies included in the current meta-analysis used the NASH CRN histological scoring system for the scoring of the liver biopsy components.[29] The two dichotomous endpoints considered were based on the currently approvable endpoints per FDA guidelines: NASH resolution without worsening of fibrosis; and fibrosis improvement without worsening of NASH.[8]

Statistical analysis

Multivariable models. Individual patient data from the 339 subjects with paired biopsy results in EMMINENCE were used to construct univariable and multivariable analysis of covariance (ANCOVA) models for the 12-month change in each histological component (steatosis, inflammation, ballooning, and fibrosis). Candidate predictors included age, sex, type 2 diabetes (T2D) history, and baseline and 12-month changes in weight and laboratory parameters (liver injury markers, cholesterols, insulin sensitivity markers, ELF, CK-18[M65]). Nonlinearity of the association between each continuous predictor and each outcome was examined by assessing the significance of the nonlinear components of a restricted cubic spline transformation for each predictor. Appropriate transformations (quadratic, cubic, linear spline, log2) were applied where deemed necessary based on the review of the Akaike's information criterion and visual examination of plots of the predicted outcomes against each predictor. Due to issues with estimating the associations when severe multicollinearity exists among predictors in the model, we excluded ALT (collinear with AST) and waist circumference (collinear with weight) as candidate predictors. Multiple imputation with 10 imputed datasets and assuming multivariate normality was used to handle missing values. The occurrence of missing data for any of the predictors was relatively low (~5% missing one or more predictor values). Rubin's algorithm was used for average estimates for predictors across the imputed datasets. Backwards selection was used for each outcome, with predictors remaining in a majority of the 10 imputed datasets at

the 0.05 significance level (>5) included in the final model. Each model was adjusted for the baseline value of the outcome. If a change from baseline predictor was included in the final model, then the baseline value for the parameter was also included in the final model.

A second multivariable model was fit for each outcome including the predictors found prognostic for any of the biopsy component changes. A multivariable ANCOVA model that included these predictors was run for NAS (the sum of ballooning, inflammation, and steatosis scores). Multivariable logistic regression models that included these predictors were also run for the binary outcomes of NASH resolution without worsening of fibrosis and fibrosis improvement without worsening of NASH.

Canonical correlation. A partial canonical correlation using individual patient data from EMMINENCE examined the correlation of changes in weight, AST, HbA1c, GGT, CK-18[M65] (taken together) with changes in ballooning, inflammation, steatosis, and fibrosis (taken together) adjusted for those baseline variables prognostic of any of the histological feature changes in the multivariable models in the 306 subjects with complete observed data for the variables involved. This analysis results in "canonical variates" that are linear combinations of the original sets of variables weighted in such a way as to maximize the correlation between them.

Meta-regressions. Random effects weighted least squares meta-regressions with inverse variance weighting were used to examine the association of changes in ALT, AST, CK-18, HbA1c, fasting insulin levels, ELF, alkaline phosphatase, and GGT with biopsy outcomes. CK-18[M30] was measured in all studies except EMMINENCE where CK-18[M65] was reported. These associations were assessed at an individual cohort level (within treatment group), as well as based on the treatment effect. Percent change from baseline in liver injury markers and biomarkers was used as the predictor when examining the associations in the individual cohorts with outcomes while the treatment ratio, the ratio of active to placebo with respect to the relative changes from baseline, was used as the predictor when assessing the association with the treatment effect on outcomes. Standardized mean changes (mean/SD) or standardized mean treatment differences (mean difference/pooled SD) were used for continuous outcomes and Freeman–Tukey double-arcsine transformed proportions or log odds ratios were used for dichotomous outcomes. Meta-regression results were computed using the metafor package available in R.[30] *P* values <0.05 were considered statistically significant.

Composite outcome. From the meta-regression results, ranks for the association with responses and treatment effects for each histologic outcome (changes in ballooning, inflammation, steatosis, and fibrosis; NASH resolution without worsening of fibrosis, and fibrosis improvement without worsening of NAS) were assigned across seven biomarkers (AST, GGT, HbA1c, insulin, CK-18, ELF, and alkaline phosphatase) for both the slope and the mediator *P* value (ranks 1–7, with 7 assigned to the biomarker with the greatest slope or lowest *P* value). An average rank across all outcomes was then assigned to each biomarker. The three biomarkers with the highest average rank, that is, with the greatest overall association with the histological outcomes, were then chosen to form a composite outcome. In order to allow comparison of effects across treatment arms and studies, an average, weighted by the biomarker's average rank across all outcomes, was computed for the standardized mean change for each treatment arm and for the standardized mean treatment difference *versus* placebo for each active treatment arm in each study.

Performance. We computed the value of the resulting novel composite outcome at baseline and 12 months for patients in EMMINENCE. The ability of baseline-adjusted 12-month changes to discriminate between patients with and without NASH resolution without worsening fibrosis, and with and without fibrosis improvement without worsening of NASH, was estimated as the area under the receiver-operator characteristic curve (AUROC) using logistic regression. For comparison, the AUROC was computed similarly for baseline-adjusted changes in other noninvasive measures including ELF,[31] Fibrotest,[32] FIB-4,[33] and Fibroscan (transient elastography) stiffness measure.[34]

SAS version 9.4 (SAS Institute, Cary, NC, USA) was used where not otherwise stated.

Results

Multivariable models. Multivariable models developed separately for each biopsy component (ballooning, steatosis, inflammation, and fibrosis) are given in Tables S1–S4. Multivariable models for each outcome including predictors found prognostic for any are shown in Table 1. Generally, inclusion of the additional covariates, or addition of MSDC-0602K dose (data not shown), did not change parameter estimates for any given outcome. Each of the models that included 12-month changes in weight, AST, HbA1c, GGT, and CK-18[M65] accounted for approximately 40% of the variance (i.e. adjusted $R^2 \approx 0.4$) in each of the biopsy score changes (Table 1). With multivariable adjustment, change in weight was significantly associated with changes in one of the four histologic features (steatosis), AST with two features (inflammation and fibrosis) and nearly significant for another one (steatosis), HbA1c with two features (ballooning and inflammation) and nearly significant for another one (steatosis), GGT with one (steatosis), and CK-18[M65] with one (ballooning) and nearly significant for another one (inflammation).

These same predictors accounted for approximately the same proportion of variance in 12-month change in NAS with an adjusted R^2 of 0.41 (Table 2). After adjustment for covariates associated with change in any individual histologic feature, 12-month changes in AST, HbA1c, and CK-18[M65] were statistically significantly associated with 12-month change in NAS. Changes in AST and HbA1c were nearly significantly associated ($P < 0.10$) with NASH resolution without worsening fibrosis, while change in AST was nearly statistically significantly associated ($P < 0.10$) with fibrosis improvement without worsening of NASH (Table 2).

Canonical correlation. The first canonical variate was found to be statistically significant ($P < 0.0001$), with a canonical correlation of about 0.49 pointing toward a positive linear

Table 1 Multivariable ANCOVA modeling results for changes in histological parameters in the EMMINENCE trial

	Ballooning			Steatosis			Inflammation			Fibrosis		
Parameter	Effect size for change of	Effect size (95% CI)	*P* value	Effect size for change of	Effect size (95% CI)	*P* value	Effect size for change of	Effect size (95% CI)	*P* value	Effect size for change of	Effect size (95% CI)	*P* value
Baseline variables												
Age (years)	57.00 *versus* 50.00	0.04 (−0.02, 0.11)	0.008	5	−0.06 (−0.11, −0.02)	0.007	5	−0.04 (−0.07, −0.01)	0.006	5	0.03 (−0.03, 0.08)	0.348
	65.00 *versus* 57.00	−0.04 (−0.13, 0.06)										
Diabetes	Yes *versus* no	−0.04 (−0.25, 0.16)	0.690	Yes *versus* No	0.08 (−0.13, 0.28)	0.451	Yes *versus* No	0.05 (−0.09, 0.18)	0.502	Yes *versus* No	0.21 (−0.04, 0.46)	0.094
Weight (kg)	5	0.00 (−0.02, 0.02)	0.768	99.06 *versus* 85.91	0.06 (−0.01, 0.13)	0.124	5	−0.00 (−0.02, 0.01)	0.543	5	0.01 (−0.02, 0.03)	0.513
				113.40 *versus* 99.06	0.00 (−0.06, 0.07)							
AST (U/L)	Doubling	0.30 (0.10, 0.51)	0.004	Doubling	0.05 (−0.15, 0.25)	0.598	Doubling	0.11 (−0.02, 0.24)	0.094	Doubling	0.51 (0.26, 0.76)	<0.001
Triglycerides (mmol/L)	Doubling	0.10 (−0.02, 0.21)	0.109	1	−0.02 (−0.07, 0.03)	0.426	1	0.01 (−0.03, 0.04)	0.666	Spline (≤2.48)	0.19 (0.00, 0.37)	0.134
										spline (>2.48)	−0.04 (−0.12, 0.04)	
HbA1c (%)	0.25	0.03 (0.01, 0.06)	0.012	0.25	0.02 (−0.01, 0.04)	0.240	0.25	0.00 (−0.01, 0.02)	0.620	0.25	0.00 (−0.03, 0.03)	0.856
GGT (U/L)	10	0.01 (−0.01, 0.02)	0.450	43.00 *versus* 31.00	−0.01 (−0.04, 0.03)	0.884	10	−0.00 (−0.01, 0.01)	0.816	10	0.01 (−0.00, 0.03)	0.161
				71.00 *versus* 43.00	−0.02 (−0.09, 0.06)							
Insulin (uIU/mL)	5	0.02 (0.01, 0.04)	0.007	5	−0.00 (−0.02, 0.01)	0.766	5	0.01 (0.00, 0.02)	0.025	5	0.00 (−0.02, 0.02)	0.862
ELF	1	0.02 (−0.09, 0.12)	0.822	1	0.07 (−0.03, 0.18)	0.172	1	0.09 (0.02, 0.16)	0.015	1	0.14 (0.01, 0.28)	0.033
CK-18[M65] (U/L)	100	0.02 (−0.00, 0.04)	0.080	100	0.01 (−0.01, 0.03)	0.571	100	0.02 (0.01, 0.03)	0.006	Spline (≤1202)	0.05 (0.00, 0.09)	0.106
										Spline (>1202)	−0.02 (−0.06, 0.02)	
Change from baseline variables												
Weight (kg)	5	0.02 (−0.05, 0.09)	0.543	5	0.08 (0.01, 0.15)	0.021	5	−0.01 (−0.06, 0.04)	0.692	5	0.03 (−0.05, 0.12)	0.478
AST (U/L)	5	0.02 (−0.01, 0.05)	0.262	5	0.02 (−0.01, 0.05)	0.155	5	0.02 (0.00, 0.04)	0.025	5	0.08 (0.04, 0.11)	<0.001
HbA1c (%)	0.25	0.03 (0.01, 0.06)	0.019	0.25	0.03 (−0.00, 0.05)	0.067	0.25	0.03 (0.01, 0.04)	0.003	0.25	0.02 (−0.02, 0.05)	0.341
GGT (U/L)	10	0.01 (−0.02, 0.04)	0.615	10	0.03 (0.00, 0.06)	0.034	10	−0.01 (−0.02, 0.01)	0.503	10	−0.01 (−0.04, 0.03)	0.706
CK-18[M65] (U/L)	100	0.02 (0.00, 0.03)	0.031	100	0.01 (−0.01, 0.02)	0.473	100	0.01 (−0.00, 0.02)	0.082	100	0.00 (−0.02, 0.02)	0.683
	Adjusted R^2: 0.3850			Adjusted R^2: 0.3390			Adjusted R^2: 0.4704			Adjusted R^2: 0.4053		

AST, aspartate aminotransferase; CI, confidence interval; CK-18, cytokeratin-18; ELF, enhanced liver fibrosis; GGT, gamma-glutamyltransferase; HbA1c, hemoglobin A1C.

Table 2 Multivariable models for histological outcomes in the EMMINENCE trial

	NAS			Resolution of NASH			Improvement in Fibrosis with no worsening of NASH		
Parameter	Effect size for change of	Effect size (95% CI)	*P* value	Effect size for change of	Odds ratio (95% CI)	*P* value	Effect size for change of	Odds ratio (95% CI)	*P* value
Baseline variables									
Age (years)	57.00 *versus* 50.00	−0.13 (−0.25, −0.01)	<0.001	5	1.00 (0.85, 1.19)	0.964	5	1.07 (0.90, 1.26)	0.445
	65.00 *versus* 57.00	−0.34 (−0.52, −0.16)							
Diabetes	Yes *versus* No	−0.01 (−0.39, 0.36)	0.939	Yes *versus* No	1.10 (0.54, 2.25)	0.789	Yes *versus* No	0.89 (0.45, 1.73)	0.724
Weight (kg)	5	0.01 (−0.03, 0.05)	0.626	5	1.02 (0.94, 1.10)	0.669	5	0.99 (0.92, 1.07)	0.877
AST (U/L)	Doubling	0.36 (−0.00, 0.73)	0.054	Doubling	0.94 (0.90, 0.97)	<0.001	1	0.97 (0.94, 1.00)	0.068
Triglycerides (mmol/L)	Spline (≤2.48)	0.27 (−0.01, 0.55)	0.172	1	0.90 (0.70, 1.17)	0.432	Spline (≤2.48)	0.60 (0.36, 1.01)	0.062
	Spline (>2.48)	−0.04 (−0.16, 0.08)					Spline (>2.48)	1.29 (0.99, 1.69)	
HbA1c (%)	0.25	0.05 (−0.00, 0.10)	0.060	0.25	0.94 (0.84, 1.05)	0.274	0.25	0.94 (0.85, 1.04)	0.217
GGT (U/L)	10	0.00 (−0.03, 0.03)	0.980	10	0.89 (0.78, 1.02)	0.087	10	0.97 (0.91, 1.05)	0.483
Insulin (uIU/mL)	5	0.03 (−0.00, 0.06)	0.092	5	0.95 (0.86, 1.05)	0.287	5	0.95 (0.87, 1.05)	0.337
ELF	1	0.16 (−0.04, 0.35)	0.114	1	0.81 (0.54, 1.22)	0.310	1	1.06 (0.73, 1.54)	0.750
CK-18[M65] (U/L)	100	0.05 (0.01, 0.09)	0.008	849.93 *versus* 569.74	0.84 (0.61, 1.16)	0.285	100	0.93 (0.84, 1.03)	0.187
				1217.80 *versus* 849.93	0.91 (0.66, 1.26)				
Change from baseline variables									
Weight (kg)	5	0.11 (−0.02, 0.24)	0.099	5	0.93 (0.73, 1.18)	0.533	5	1.03 (0.82, 1.28)	0.825
AST (U/L)	5	0.06 (0.00, 0.11)	0.040	5	0.85 (0.70, 1.03)	0.096	5	0.89 (0.74, 1.07)	0.210
HbA1c (%)	0.25	0.08 (0.03, 0.13)	0.003	0.25	0.88 (0.77, 1.01)	0.080	0.25	0.94 (0.84, 1.06)	0.336
GGT (U/L)	10	0.02 (−0.03, 0.07)	0.383	10	1.00 (0.80, 1.25)	0.996	10	0.97 (0.84, 1.11)	0.649
CK-18[M65] (U/L)	100	0.04 (0.01, 0.07)	0.012	100	0.98 (0.90, 1.05)	0.513	100	0.92 (0.82, 1.03)	0.150
	Adjusted R^2: 0.4118			C-Statistic: 0.8207			C-Statistic: 0.7593		

AST, aspartate aminotransferase; CI, confidence interval; CK-18, cytokeratin-18; ELF, enhanced liver fibrosis; GGT, gamma-glutamyltransferase; HbA1c, hemoglobin A1C; NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis.

correlation between the two sets of changes (12-month changes from baseline in biomarkers and in biopsy results). Standardized canonical coefficients (the weights used to maximize the correlation) and correlations of each of the component variables with the canonical variate suggest that changes in AST, HbA1c, and CK-18[M65] had the strongest influence on the first canonical variate for the biomarker, while all four biopsy outcome variables appeared to contribute more or less equally to their canonical variate, with the strongest influence observed for changes in inflammation (Table S5). AST, HbA1c, and CK-18[M65] also had the highest correlations with the biopsy canonical variate.

Meta-regressions. Seventeen NASH clinical trials, comprising 3717 patients, were included in the meta-analyses (Figure S1). Study characteristics are described in Table S6. Total sample sizes ranged from 47 to 931 patients and follow-up from 6 to 22 months. Laboratory changes from baseline and changes in histological outcomes for each study are given in Tables S7–S8.

Figure 1 provides an example of meta-regressions evaluating the association between biomarker changes and changes in biopsy findings (in this case, fibrosis improvement) and between treatment effects on biomarkers and treatment effects on histology. All meta-regression results are provided in Figures S2–S15. A decrease in insulin was associated with a decrease in steatosis (Figure S4), while a decrease in GGT was associated with a decrease in inflammation (Figure S6). Decreases in AST and HbA1c were associated with decreases in ballooning, inflammation, steatosis, and fibrosis, while a decrease in CK-18[M30 or M65] was associated with a decrease in ballooning, inflammation, and fibrosis, though not steatosis (Figures S2, S4, S6 and S8). The results for ALT followed those of AST. Other biomarker responses were not associated with histologic score changes.

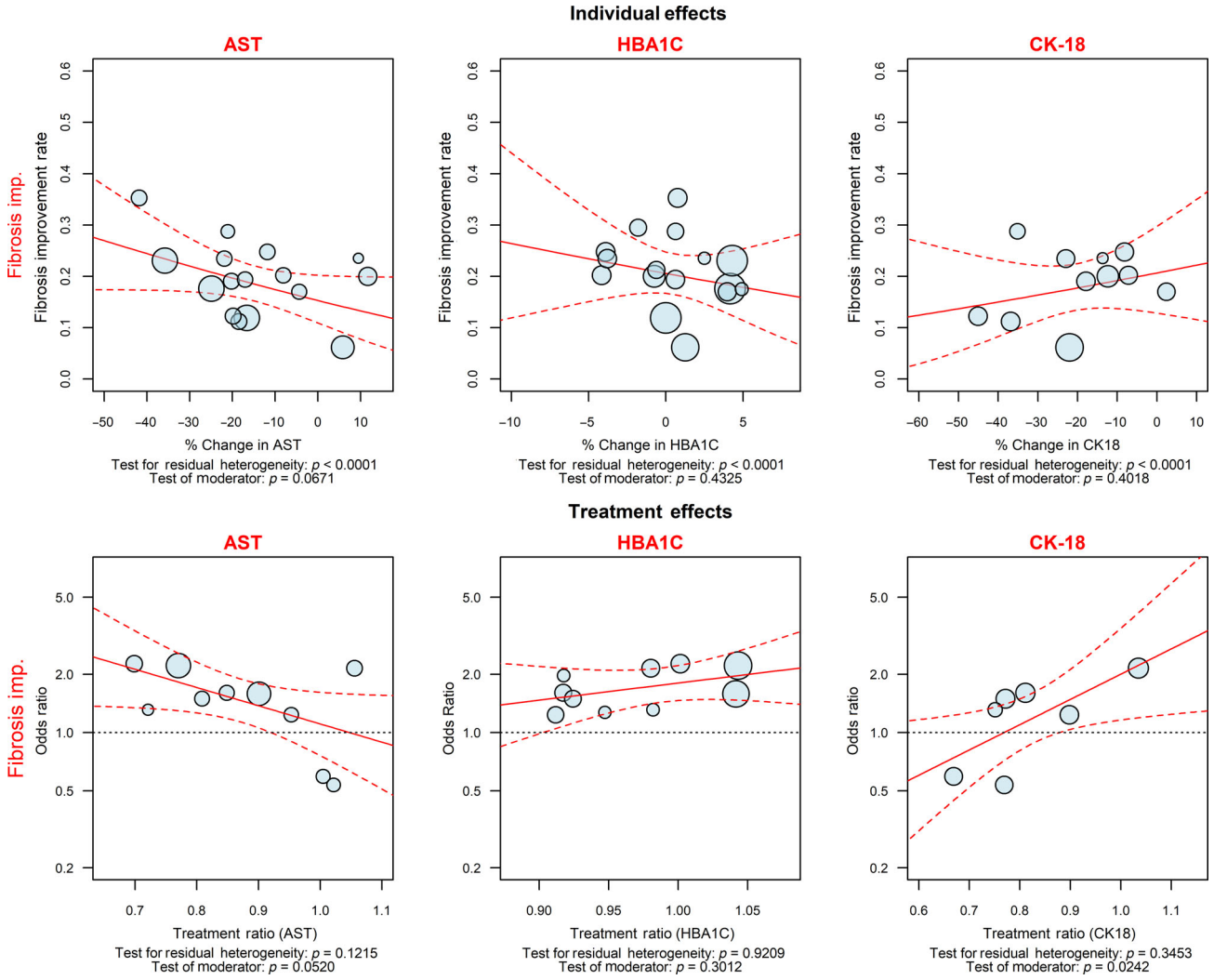

Figure 1 Association of changes and treatment ratio in aspartate aminotransferase (AST), hemoglobin A1C (HbA1c), and cytokeratin-18 (CK-18) with rates and treatment effects of the histological endpoint fibrosis improvement without worsening of non-alcoholic steatohepatitis (NASH) in 17 NASH clinical trials.

Table 3 Ranking of biomarkers with respect to strengths of associations of biomarker changes with changes in histological scores and outcomes, and with treatment effects on biomarker changes and treatment effects on histological outcomes, found in the meta-regressions

Histological feature	Association	Meta-regression result	AST	GGT	HbA1c	Insulin	CK-18	ELF	Alk phos
Inflammation	Individual	Slope	0.0182	0.0081	0.0327	0.0043	0.0100	0.0699	0.0010
		Slope rank	5	3	6	2	4	7	1
		P value	0.0000	0.0008	0.0260	0.2485	0.0003	0.1752	0.8976
		P value rank	7	5	4	2	6	3	1
	Treatment effect	Slope	3.0443	1.9509	−6.822	−0.5055	3.3492	21.472	−2.060
		Slope rank	5	4	1	3	6	7	2
		P value	0.0463	0.1492	0.2345	0.4841	0	0.0548	0.1688
		P value rank	6	4	2	1	7	5	3
Ballooning	Individual	Slope	0.004	0.0032	0.0317	0.0039	0.0064	0.005	−0.0056
		Slope rank	4	2	7	3	6	5	1
		P value	0.0124	0.1323	0.0129	0.1235	0.045	0.2599	0.1797
		P value rank	7	3	6	4	5	1	2
	Treatment effect	Slope	1.0422	−0.1795	−0.9912	−0.1555	0.7172	8.6291	−0.2175
		Slope rank	6	3	1	4	5	7	2
		P value	0.0348	0.751	0.5581	0.528	0.2612	0.4405	0.6702
		P value rank	7	1	3	4	6	5	2
Steatosis	Individual	Slope	0.0155	0.0054	0.0414	0.0076	0.0066	−0.034	−0.0077
		Slope rank	6	3	7	5	4	1	2
		P value	0.0034	0.1047	0.009	0.0445	0.165	0.5143	0.3476
		P value rank	7	4	6	5	3	1	2
	Treatment effect	Slope	2.4744	−0.6187	−1.581	0.2354	2.0742	−3.847	0.1169
		Slope rank	7	3	2	5	6	1	4
		P value	0.0002	0.437	0.6538	0.5789	0.0043	0.7306	0.8974
		P value rank	7	5	3	4	6	2	1
Fibrosis	Individual	Slope	0.0122	0.0051	0.0315	0.0024	0.0079	0.0383	0.0008
		Slope rank	5	3	6	2	4	7	1
		P value	0.0001	0.1051	0.0132	0.4252	0.0035	0.43	0.8703
		P value rank	7	4	5	3	6	2	1
	Treatment effect	Slope	1.204	1.1268	−2.772	−0.348	1.2725	14.251	−0.6058
		Slope rank	5	4	1	3	6	7	2
		P value	0.0424	0.0406	0.1012	0.1575	0.0461	0.1988	0.1136
		P value rank	6	7	4	2	5	1	3
NASH resolution	Individual	Slope	−0.005	−0.0011	−0.0226	−0.0032	−0.0016	−0.0081	−0.0035
		Slope rank	5	1	7	3	2	6	4
		P value	0.0234	0.3501	0.0001	0.1043	0.5323	0.4294	0.1036
		P value rank	6	3	7	4	1	2	5
	Treatment effect	Slope	−2.5	−0.8787	−2.047	0.623	−2.631	−8.107	1.0893
		Slope rank	5	3	4	2	6	7	1
		P value	0.0001	0.369	0.3492	0.6326	0.3046	0.4392	0.4535
		P value rank	7	4	5	1	6	3	2
Fibrosis improvement	Individual	Slope	−0.0028	−0.0015	−0.0068	−0.0017	0.0018	−0.0109	−0.0031
		Slope rank	4	2	6	3	1	7	5
		P value	0.0671	0.0992	0.4325	0.4725	0.4018	0.1686	0.19
		P value rank	7	6	2	1	3	5	4
	Treatment effect	Slope	−2.167	−0.5556	2.0349	0.9367	3.0063	1.1362	0.1869
		Slope rank	7	6	2	4	1	3	5
		P value	0.052	0.4688	0.3012	0.2811	0.0242	0.9042	0.8321
		P value rank	6	3	4	5	7	1	2
Average slope/ *P* value rank			6.00	3.58	4.21	3.12	4.67	4.00	2.42

AST, aspartate aminotransferase; CK-18, cytokeratin-18; ELF, enhanced liver fibrosis; GGT, gamma-glutamyltransferase; HbA1c, hemoglobin A1C; NASH, non-alcoholic steatohepatitis.

Greater treatment effects (i.e. lower treatment ratios) on AST were associated with greater treatment effects (i.e. lower mean treatment differences in histology scores) on ballooning, steatosis, inflammation, and fibrosis (Figures S3, S5, S7 and S9). Treatment effects on HbA1c were not associated with treatment effects on any of these histology scores, while greater

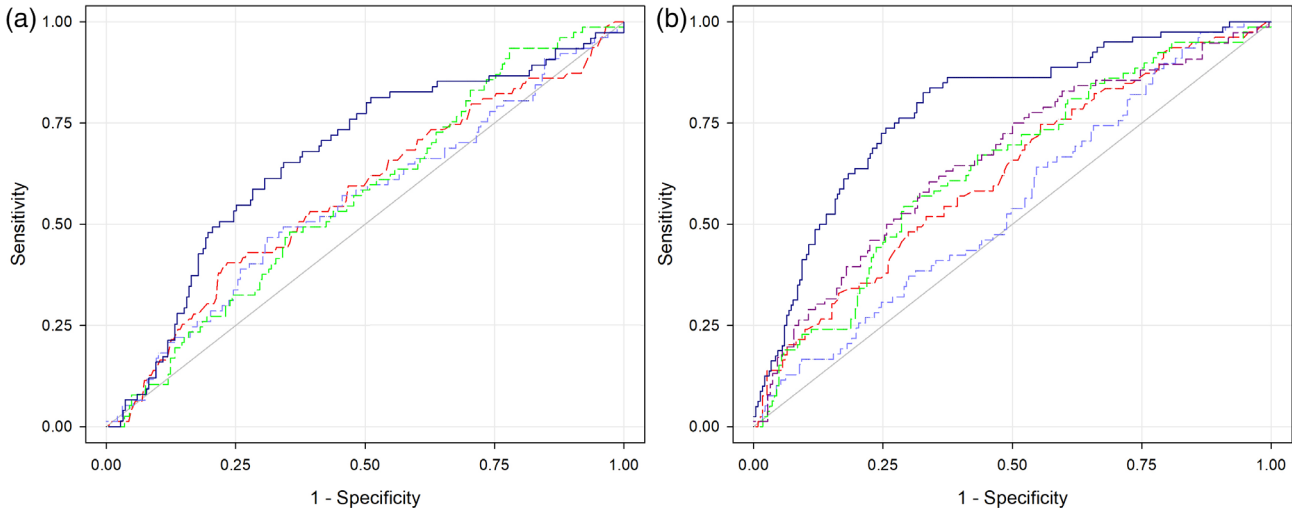

Figure 2 Receiver-operator characteristic curves for associations of baseline-adjusted 12-month changes with (a) fibrosis improvement without worsening of non-alcoholic steatohepatitis (NASH); (-----), Weighted-Z (area under the curve [AUC]: 0.755); (-----), enhanced liver fibrosis (ELF) (AUC: 0.572); (-----), FibroTest (AUC: 0.557); (-----), Fib-4 (AUC: 0.570); (-----), Fibroscan (AUC: 0.668) and (b) NASH resolution without worsening of fibrosis in the EMMINENCE trial; (-----), Weighted-Z (AUC: 0.788); (-----), ELF (AUC: 0.625); (-----), FibroTest (AUC: 0.554); (-----), Fib-4 (AUC: 0.643); (-----), Fibroscan (AUC: 0.663).

treatment effects on CK-18[M30 or M65] were associated with greater treatment effects on steatosis, inflammation, and fibrosis but not with ballooning.

Changes in CK-18[M30 or M65] but not changes in AST or HbA1c were associated with changes in NAS (Figure S10). Changes in AST and HbA1c but not CK-18 were associated with the rate of NASH resolution (Figure S12). None of these three biomarkers was associated with fibrosis improvement without worsening of NASH, although the change in AST was nearly so (Fig. 1, Figure S14). Only the treatment effect on AST was associated with treatment effect on NASH resolution without worsening of fibrosis (Figure S13), and only CK-18 with fibrosis improvement without worsening of NASH (Fig. 1, Figure S15).

Composite endpoint. Based on the meta-regression results, the three biomarkers best associated with liver histology improvements overall were (in descending order) AST, CK-18[M30 or M65], and HbA1c with average ranks across all outcomes of 6.00, 4.67, and 4.21, respectively (Table 3). The weighted average (0.403 × AST, 0.314 × CK-18, 0.283 × HbA1c) of changes in these three biomarkers may be considered reflective of expected overall effects on histologic parameters.

The five interventions with the largest treatment effects on this new surrogate endpoint were pioglitazone, Aramachol, resmetiron, MSDC-0602K, and liraglutide. In two studies [ARREST (Aramchol)[20] and Madrigal phase 2b (resmetiron)[19]], treatment effects relative to placebo were driven primarily by large adverse changes in the placebo group, while in other studies such as the pioglitazones studies of Cusi[15] and Belfort,[14] the LEAN study of liraglutide,[17] ENCORE-NF (Emricasan),[22] and FLINT (obeticholic acid)[10] large improvements in the placebo group were observed, suggesting an overall trend to improvement in all patients enrolled in the study (Table S9).

In the pooled treatment groups in EMMINENCE, the AUROC for the baseline-adjusted 12-month change in the AST/HbA1c/CK-18 composite was the highest among those examined (Fig. 2a): 0.7553 for fibrosis improvement without worsening of NASH and 0.7880 for NASH resolution without worsening of fibrosis (Fig. 2b). The measure with the highest AUROCs among the others examined was Fibroscan stiffness for both fibrosis improvement without worsening of NASH (0.6679) and for NASH resolution without worsening of fibrosis (0.6627).

Discussion

The results of the present analysis suggest that in patients with NASH treated with new interventions, a weighted score of AST, CK-18, and HbA1c changes is associated with histologic improvement in NASH. This result has been seen in both analysis of the EMMINENCE study and a meta-analysis of 17 studies including 3717 patients.

NASH is a substantial disorder with high prevalence leading to significant adverse outcomes including cirrhosis, hepatocellular carcinoma, and need for liver transplantation.[3,4] Its combined high prevalence and adverse effects have therefore very important consequences on the health of patients as well as significant economic effects on health systems. Therefore, developing new therapies for NASH is of utmost importance. However, such development of new interventions for NASH has been hindered by the need to perform complex and expensive studies with "paired" liver biopsies, that is, liver biopsies before and after 1–1.5 years of treatment, for the initial assessment of the efficacy of new treatments. Beyond being very complex to perform and expensive, "paired" liver biopsies studies are fraught with significant problems. First, liver biopsies have some risks leading to adverse events.[28] Second, they are limited by sampling errors, as NASH is not uniformly present at the same severity

throughout the liver.[26] Finally, in a post hoc analysis of the EMMINENCE study, we have found that liver biopsy interpretation is limited.[27]

As of now, no drug or intervention has been approved for the treatment of NASH. Some of this lack of progress may relate to the complexity, cost, and lack of accuracy of "paired" biopsies studies. Hence, in the current analysis, we sought to identify simple, noninvasive measures to assess NASH improvements. Our analysis in both the EMMINENCE study (using either multivariate regression or canonical models) and in a meta-analysis of 17 studies, changes in AST, CK-18, and HbA1c are best associated with overall histological improvements in NASH.

The weighted average of standardized changes in AST, CK-18, and HbA1c was used to assess the changes in the placebo and active arms of the studies included in the meta-analysis. The results presented in Table S10 suggest that many of these interventions are effective in improving this combined score, and hence potentially beneficial in NASH. Some of the interpretation is limited by unstable placebo effects—in some studies, the placebo patients improved substantially, suggesting that other beneficial interventions may have been implemented in parallel to the study drug during the follow-up period, while in other studies, the placebo patients showed substantial worsening, suggesting that some of the suggested treatment effects may have been related to worsening in the placebo arm. This in turn, in smaller studies, may have been due to chance. However, the use of the proposed score enables better comparative qualitative assessment of treatment effects and can therefore facilitate a simple tool to assess the efficacy of therapies in early NASH studies, avoiding the complexity and limitations of "paired" liver biopsy studies.

The baseline-adjusted change in the AST/HbA1c/CK-18 composite score better discriminated between histological responders and nonresponders in patients in the EMMINENCE trial than did changes in other biochemical and imaging markers, even for fibrosis improvement without worsening of NASH. This finding may not be surprising as these other measures were specifically developed to detect fibrosis and not change in NASH over time.[35]

The current analysis is limited by the variables available for analysis in the EMMINENCE study and the 17 studies identified, as well as by the heterogeneity of trial settings and how histopathologist(s) read biopsies. It is possible that other more accurate measures for NASH resolution exist. The combined score of AST, HbA1, and CK-18 was found to be associated with biopsy determined NASH improvement. Hence, it is limited by the lack of reliability of liver biopsy in identifying NASH and changes in NASH, and further research into methods to increase this reliability is needed. The baseline-adjusted change in the weighted average of standardized values is proposed as a comparative measure of treatment response in the context of a clinical trial, where the magnitude of observed changes is gauged relative to the mean and variance in the study population. A composite biomarker reflecting the liver histology of an individual patient might be developed but would require access to larger clinical databases. Any such composite biomarker would ultimately require validation against clinical events.

In conclusion, changes in AST, HbA1c, and CK-18 were identified in both covariate-adjusted models of the EMMINENCE study and meta-analysis of 17 studies including 3717 patients to be associated with histological improvement in NASH. A score combining these biomarkers might be used for the assessment of the efficacy of new interventions for NASH in early development, reducing the dependence of these studies on complex, expensive, and unreliable "paired" liver biopsies. Further development of such a composite biomarker is warranted.

Conflict of Interest

Beth A Davison, Gad Cotter, and Christopher Edwards report grants from Cirius Therapeutics, Inc. during the study's conduct. Rohit Loomba serves as a consultant or advisory board member for Arrowhead Pharmaceuticals, AstraZeneca, Bird Rock Bio, Boehringer Ingelheim, Bristol-Myer Squibb, Celgene, Cirius, CohBar, Conatus, Eli Lilly, Galmed, Gemphire, Gilead, Glympse bio, GNI, GRI Bio, Intercept, Ionis, Janssen Inc., Merck, Metacrine, Inc., NGM Biopharmaceuticals, Novartis, Novo Nordisk, Pfizer, Prometheus, Sanofi, Siemens, and Viking Therapeutics. In addition, his institution has received grant support from Allergan, Boehringer-Ingelheim, Bristol-Myers Squibb, Cirius, Eli Lilly and Company, Galectin Therapeutics, Galmed Pharmaceuticals, GE, Genfit, Gilead, Intercept, Grail, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, NuSirt, Pfizer, pH Pharma, Prometheus, and Siemens. He is also co-founder of Liponexus, Inc. Rohit Loomba also receives funding support from NIEHS (5P42ES010337), NCATS (5UL1TR001442), NIDDK (R01DK106419, P30DK120515), and DOD PRCRP (CA170674P2). Stephen A Harrison reports grants from Cirius Therapeutics, during the conduct of the study; grants, personal fees and other from Galectin, Genfit, Madrigal, Metacrine, NGM Bio, grants and personal fees from 3VBio, Axcella, Cirius Therapeutics, Contravir, CymaBay, Galmed, Gilead, BMS, Hightide, Intercept, Immuron, Novartis, Novo Nordisk, Pfizer, Second Genome, Tobira/Allergan, grants and other from NorthSea, grants from Conatus, personal fees and other from Akero, Altimmune, Blade Therapeutics, personal fees from CiVi Biopharma, Echosens, Gelesis, CLDF, Consynance, Corcept, HistoIndex, Indalo, Innovate, IQVIA, Lipocine, Medpace, Perspectum, Prometheus, Poxel, Prometic, Fortress Bio, Terns, Viking, outside the submitted work. Naim Alkhouri reports grants from Cirius Therapeutics during the conduct of the study; and grants from Albireo, Ackero, Boehringer Ingelheim, Bristol-Myers Squibb, Galmed, Genfit, Hanmi, Inventiva, Madrigal, MedImmune, Novartis, Novo Nordisk, Pfizer, Poxel, and Zydus; grants and personal fees from Allergan, Gilead, Intercept; AbbVie, Alexion and Eisai and personal fees from Exelixis and Salix outside the submitted work. Howard C Dittrich reports personal fees from Cirius Therapeutics, Inc., during the conduct of the study. In addition, there is a patent MSDC-0602K method of use pending. Gary G Koch is the principal investigator of a biostatistical agreement between Momentum Research, Inc. and the University of North Carolina at Chapel Hill, and that agreement provided the structure for his activity for this article. He is also the principal investigator of many such biostatistical agreements with other biopharmaceutical sponsors, including AbbVie, Amgen, Arena, AstraZeneca, Eli Lilly & Co., Forest Research Institute (Allergan), GlaxoSmithKline, Merck, Novartis, Otsuka, Pfizer, and Sanofi, although his activities for those sponsors are not related to the content of this article. Information concerning all biostatistical agreements for which Gary Koch is the principal investigator is publicly available through the University of North Carolina at Chapel Hill.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Appendix S1 Supporting Information.