Am J Physiol Gastrointest Liver Physiol 316: G25–G31, 2019.
First published August 30, 2018; doi:10.1152/ajpgi.00158.2018.

RESEARCH ARTICLE | *Inflammation, Immunity, Fibrosis, and Infection*

Assessment of liver fibrosis progression and regression by a serological collagen turnover profile

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Submitted 8 May 2018; accepted in final form 20 August 2018

Karsdal MA, Hjuler ST, Luo Y, Rasmussen DG, Nielsen MJ, Holm Nielsen S, Leeming DJ, Goodman Z, Arch RH, Patel K, Schuppan D. Assessment of liver fibrosis progression and regression by a serological collagen turnover profile. *Am J Physiol Gastrointest Liver Physiol* 316: G25–G31, 2019. First published August 30, 2018; doi:10.1152/ajpgi.00158.2018.—There is a need for noninvasive biomarkers that can identify patients with progressive liver fibrosis and monitor response to antifibrotic therapy. An equally important need is identification of patients with spontaneous fibrosis regression, since they may not need treatment nor be included in clinical studies with fibrosis as end point. Circulating biomarkers, originating from defined fragments of the scar tissue itself, may serve as valuable tools for this aspect of precision medicine. We investigated a panel of serological collagen formation and degradation markers to identify patients likely to regress or progress in absence of a therapeutic intervention. Plasma samples from patients with moderate-stage hepatitis C receiving placebo treatment in a phase II trial of the peroxisome proliferator-activated receptor agonist farglitazar were included. The patients had matched liver biopsies at baseline and 52 wk of follow-up. Serological biomarkers of collagen formation (PRO-C3, PRO-C4, PRO-C5) and collagen degradation (C3M, C4M, and C6M) were analyzed. Logistic regression analysis including PRO-C3 and C6M identified subjects with progressive liver fibrosis with an AUROC of 0.91 ($P < 0.0001$) and positive and negative predictive values (PPV/NPV) of 75.0%/88.6%. Low levels of PRO-C5 predicted a spontaneous regression phenotype, with an odds ratio of 33.8 times higher compared with patients with high levels ($P < 0.0025$) with an AUROC of 0.78 ($P < 0.0001$) and PPV/NPV of 60.0%/95.7%. Two collagen fragments (PRO-C3 and C6M) identified liver fibrosis progressors, and one collagen fragment (PRO-C5) identified liver fibrosis regressors. These biomarkers may improve patient stratification and monitor treatment efficacy in studies with fibrosis as clinical end point.

NEW & NOTEWORTHY In this study we report two biomarkers of collagen fragments (PRO-C3 and C6M) that are able to identify liver fibrosis progressors while one biomarker (PRO-C5) identified liver fibrosis regressors. In particular, we present three noninvasive biomarkers that can be used to identify patients with progressive liver fibrosis, monitor response to antifibrotic therapy, and also identify the spontaneous liver fibrosis regression phenotype.

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basment membrane; biomarker; fibrogenesis; fibrolysis; procollagen; serum

INTRODUCTION

Cirrhosis and its complications are linked to a highly increased morbidity and mortality and represent the most relevant clinical end point in patients with chronic liver diseases (CLD) (32, 47). Therefore, inhibition of fibrosis progression to cirrhosis or regression of (compensated) cirrhosis have become the primary end points of most clinical trials in patients with CLD. In light of highly effective antiviral therapies, the leading causes of CLD in need of antifibrotic therapies are currently nonalcoholic steatohepatitis (NASH) (41), alcoholic steatohepatitis (ASH), and alcoholic cirrhosis but also rarer congenital, autoimmune, and drug-induced liver diseases (37). Moreover, between 10 and 20% of chronic hepatitis B and C patients on novel highly effective antiviral therapies still experience fibrosis progression or progression from compensated to decompensated cirrhosis, despite effective virus elimination (HCV) or suppression (HBV), stressing the need for their early identification and treatment to prevent fibrosis progression and induce regression of compensated cirrhosis (4, 21). However, even in face of the obvious clinical need and recent key advances in clinical research (45), there are still no approved antifibrotic therapies (38, 40, 44).

In most patients, liver scarring is a protracted process that is largely asymptomatic in earlier stages and often needs decades to reach the cirrhotic stage. Consequently, clinical studies with histological or clinical end points need to be long term with large patient numbers, with an uncertain outcome even after many years and extensive investment. Such studies have therefore been unattractive for drug developers. Moreover, a major challenge for clinical development is identifying those patients who are more likely to progress vs. those who spontaneously regress (1, 34). Here, noninvasive identification of nonprogressors or spontaneous regressors would avoid unnecessary exposure to experimental treatment and dramatically reduce healthcare costs (11, 41).

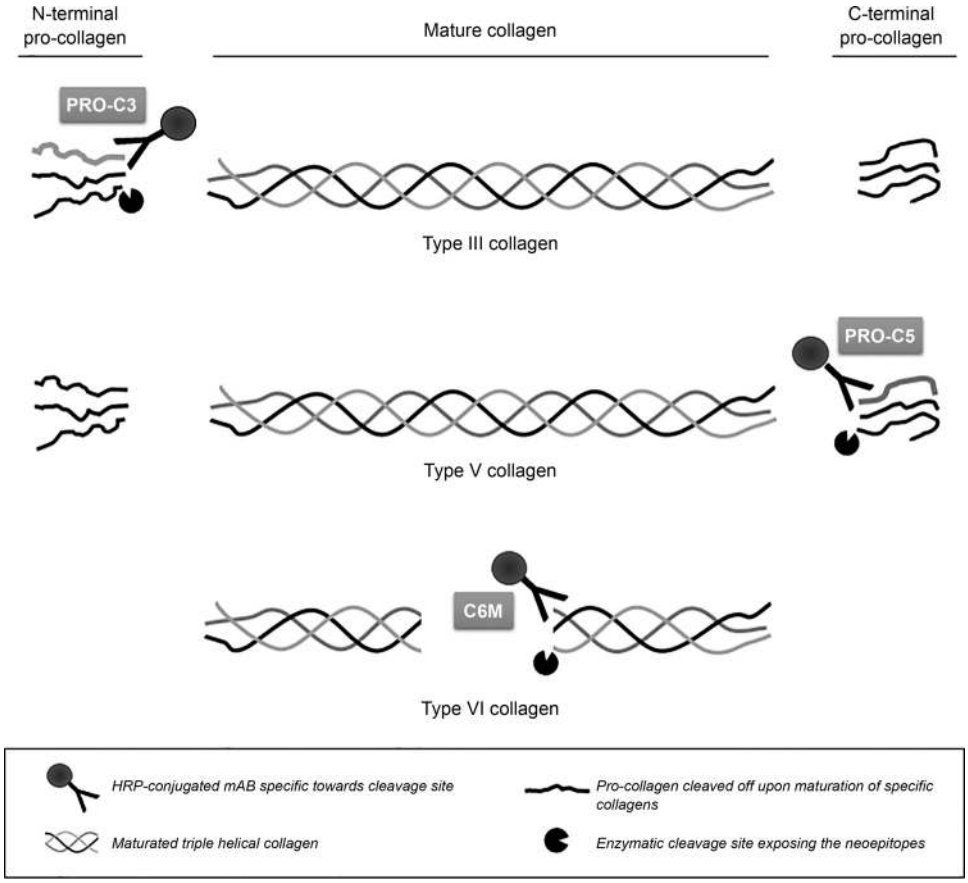

Fig. 1. Schematic representation of the collagen type III, V, and VI, with the epitopes detected by the PRO-C3, PRO-C5, and C6M assay antibodies. The PRO-C3 assay detects the cleavage site epitope generated by ADAMTS-2 cleavage of the propeptide, resulting in the release of the propeptide from the collagen sequence, allowing the mature collagen to be incorporated in the extracellular matrix (ECM). The PRO-C5 assay detects the epitope generated by BMP-1, resulting in the release of the propeptide from the collagen sequence, allowing the mature collagen to be incorporated into the ECM. C6M detects an epitope internally in the type VI collagen triple helix region that is exposed by multiple MMPs when the collagen structure is degraded.

A cirrhotic liver contains up to 10 times more collagen than a healthy liver (14). Hence we recently developed a collagen formation and degradation panel of biomarkers, including both interstitial matrix and basement membrane collagens, to noninvasively assess fibrogenesis and fibrolysis, which may allow rapid assessment of efficacy on treatment with antifibrotic therapies and finally a personalized therapeutic approach (11, 41). We showed that degradation and formation markers of type IV collagen correlate with liver fibrosis and inflammation in patients with chronic hepatitis B and C (24). Moreover, using the fibrogenesis marker PRO-C3, we could identify patients who were fast progressors as well as predict and monitor their favorable therapeutic response in a trial of HCV-infected patients treated with farglitazar and in patients with type 2 diabetes treated with two different glitazones (13, 28). However, additional collagen biomarkers and collagen marker combinations may both refine those findings and at the same time may allow identification of fibrosis regressors.

In this study, we expanded our serological collagen biomarker panel to include fingerprint markers of collagen degradation, to identify patients likely to regress who would consequently not be in need of antifibrotic therapy. Moreover, we refined the progression panel to be able to identify progressors that will be more likely to respond to therapy with an even higher accuracy.

METHODS

Plasma samples. The farglitazar study was a phase II, randomized, double-blinded, placebo-controlled multicenter study to determine the antifibrotic effect of a highly potent and specific peroxisome proliferator-activated receptor (PPAR)-γ agonist in subjects with chronic hepatitis C with intermediate-stage fibrosis who had not responded to interferon-based regimens (NCT00244751). A baseline biopsy was taken <120 days before treatment to confirm the presence of intermediate-stage fibrosis (Ishak stage 2, 3, or 4) as determined by a single expert pathologist. A second biopsy was obtained at the end of the study. The study was conducted in accordance with the ethics principles of the Declaration of Helsinki and was consistent with local regulatory authorities in each country. All patients provided written informed consent. Blood samples for biomarker evaluation were collected at baseline and after 52 wk. Overall, the two doses of farglitazar did not show antifibrotic efficacy after 52 wk of treatment based on histological fibrosis stage and morphometrical collagen deposition (22). In a subpopulation including 194 patients, we have previously shown that formation of type III collagen assessed by the PRO-C3 assay could predict progressors of fibrosis in this study (28). Moreover, in a post hoc analysis, we have previously demonstrated that patients with high baseline levels of PRO-C3, i.e., active fibrogenesis, showed significant responses to treatment with farglitazar, had treatment effect as moirrored by a 20% PRO-C3 reduction, and were protected from liver fibrosis progression compared with patients with lower PRO-C3 levels (13). Consequently, farglitazar-treated patients were excluded for the current analyses, and samples used were from the placebo arm consisting of 52 patients with available plasma and two biopsies.

Biomarker assessments. Plasma markers of type III, IV, and V collagen formation (PRO-C3, PRO-C4, and PRO-C5, respectively) and type III, IV, and VI collagen degradation (C3M, C4M, and C6M, respectively) were established using competitive ELISA and thoroughly validated as to reproducibility and clinical performance as previously described (2, 10, 18–20, 25, 26, 35, 48) (Fig. 1). Concen-

trations of aspartate aminotransferase (AST) were assessed using a standard assay and method.

Statistical analysis. Patients were stratified based on the calculated delta Ishak score that was calculated as the change from baseline liver biopsy to the biopsy at 52 wk. Progressors were defined as patients who increased by one or more Ishak stages and regressors as patients who decreased by one or more Ishak stages. Differences between groups were calculated by one-way analysis of variance (ANOVA) for continuous variables or by Chi-squared analysis for categorical variables. AUROC analyses were performed to define patients with high or low baseline levels of PRO-C3, PRO-C4, PRO-C5, C3M, C4M, and C6M based on the Youden index when separating progressors or regressors from the rest of the population. The Youden index describes the maximal potential effectiveness of a biomarker by identifying the optimal cutoff for differentiating groups of patients when weighing both specificity and sensitivity on the receiver operating characteristics (ROC) as described previously (49). The progressors and regressors were separated on the basis of the cutoff values in categories below/above cutoff (0 or 1), and the categorical data were analyzed using logistic regression using forward elimination, resulting in area under the curve (AUC) and classification table. The classification table was used to calculate the odds ratio for being progressor or regressor.

To test the robustness of the findings, we used bootstrapping and leave-one-out cross validation (LOOCV). With bootstrapping, 1,000 replicated data sets of random patients with replacement were generated from the study subjects and tested with the model to evaluate the performance. LOOCV used one sample from the cohort of patients as the validation data and the remaining observations as the training data. This procedure was repeated such that each of the patients was used one time as the validation data. The overall mean accuracy of the prediction model was calculated as the proportion of all correct predictions. Bootstrap resampling and LOOCV analysis were carried out in R.

Statistical calculations were carried out using MedCalc version 14.8.1 or R (version 3.2.4; The R Foundation). Graphs were plotted using GraphPad Prism 7.4. P values <0.05 were considered statistically significant.

RESULTS

In the 52 patients with two biopsies in the placebo arm of the subpopulation of the farglitazar study, fibrosis progressed in 11 patients, was stable in 36 patients, and regressed in 5 patients based on delta Ishak scores. The characteristics of the patients in the placebo group when separated into phenotypes (regressor, stable, progressor) can be seen in Table 1. AST, PRO-C3, and C6M were the only markers associated with phenotype. We generated cutoff values using the Youden index from AUROC analyses for dividing patients into high or low biomarker level at baseline in regard to being progressor or regressor of fibrosis. The identified cutoff levels can be seen in Table 2.

Progressors. Patients were stratified based on cutoff levels calculated using the Youden index, and a logistic regression model was used to calculate odds ratios for being progressors of fibrosis. Using forward elimination, only high baseline PRO-C3 and C6M were independent predictors of fibrosis progression. These markers predicted progressors of fibrosis with individual odds ratios of 19.4 ($P = 0.003$) and 11.6 ($P = 0.011$) for patients with baseline levels of PRO-C3 >22.4 and C6M >11.6 ng/ml, respectively, compared with patients with biomarker levels below these values. This indicates that PRO-C3 is a stronger predictor of progression compared with C6M. The model itself gave an odds ratio of 23.4 ($P < 0.001$), as depicted in Fig. 2*A*. The diagnostic performance for detecting progressors with this model is described by an AUC = 0.91, sensitivity = 54.5%, specificity = 95.1%, and positive and negative predictive values (PPV/NPV) of 75.0 and 88.6%, respectively (Fig. 2*B*). Based on unbiased selection for accurately identifying the progressors, the associated criterion values of the ROC curve are listed in Table 3.

Table 1. *Baseline characteristics of the 52 placebo-treated patients stratified for their phenotype (progressor, stable, regressor)*

	Regressors	Stable	Progressors	P Value
n	5	36	11	
Age, yr	50.2 (3.90)	50.9 (6.65)	48.7 (6.02)	NS
Men, *n* (%)	5 (100)	24 (66.7)	7 (63.6)	NS
BMI, kg/m^2	30.9 (4.86)	28.9 (5.27)	31.2 (6.01)	NS
Baseline Ishak score	2.8 (0.45)	2.5 (0.70)	2.8 (0.87)	NS
Delta Ishak score	−1.0 (0.0)	0.0 (0.0)	1.3 (0.47)	<0.001
AST, IU/l	42.6 (21.44)	60.0 (42.01)	112.2 (72.12)	0.007
Fibrotest, IU	0.6 (0.23)	0.6 (0.26)	0.7 (0.24)	NS
PRO-C3, ng/ml	14.6 (5.20)	19.4 (11.65)	32.5 (14.21)	0.004
PRO-C4, ng/ml	214.6 (112.61)	212.3 (83.21)	228.7 (72.34)	NS
PRO-C5, ng/ml	341.6 (194.45)	381.3 (128.30)	376.4 (164.56)	NS
C6M, ng/ml	7.4 (2.26)	9.6 (3.99)	12.4 (3.37)	0.034
C4M, ng/ml	42.3 (14.15)	48.9 (17.98)	59.8 (19.61)	NS
C3M, ng/ml	15.9 (5.57)	19.1 (7.39)	20.3 (7.76)	NS

Values are means (SD); *n*, number of patients. BMI, body mass index; AST, aspartate aminotransferase. P values are calculated by 1-way analysis of variance for continuous variables or by χ^2-test for categorical variables. NS, nonsignificant P value.

To test the robustness of the findings, we did internal cross-validation of the model using bootstrap resampling and LOOCV. This revealed a stable AUC of 0.90 and 0.86 for bootstraping and LOOCV analysis, respectively. The overall accuracy for the model was 73.1%, i.e., 38 out of 52 patients were correctly classified.

Regressors. Patients were also stratified based on specific cutoff levels for regressors, and a logistic regression model was used to calculate odds ratios for being regressors of fibrosis. The best model for describing regressors included PRO-C5 as a single marker. This resulted in a odds ratio for being a regressor of fibrosis of 33.8 times higher ($P < 0.0025$) for patients with baseline levels of PRO-C5 $\leq$239.9 ng/ml compared with patients with biomarker levels above this value, as depicted in Fig. 3*A*. The diagnostic performance for detecting regressors with this model is described by an AUC = 0.78, sensitivity = 60.0%, specificity = 95.7%, and PPV/NPV of 60.0%/95.74% compared with patients with PRO-C5 levels $>$239.9 ng/ml (Fig. 3*B*). Based on unbiased selection for

Table 2. *Associated cutoffs used for the prediction models*

Biomarker	Progressors vs. Rest	Regressors vs. Rest
n	NS11/41	NS5/47
PRO-C3, ng/ml	>22.4	<15.1
PRO-C4, ng/ml	>172.4	<149.8
PRO-C5, ng/ml	<264.5	<239.9
C3M, ng/ml	>16.5	<16.4
C4M, ng/ml	>57.9	<34.2
C6M, ng/ml	>11.6	<9.9

n, Number of patients.

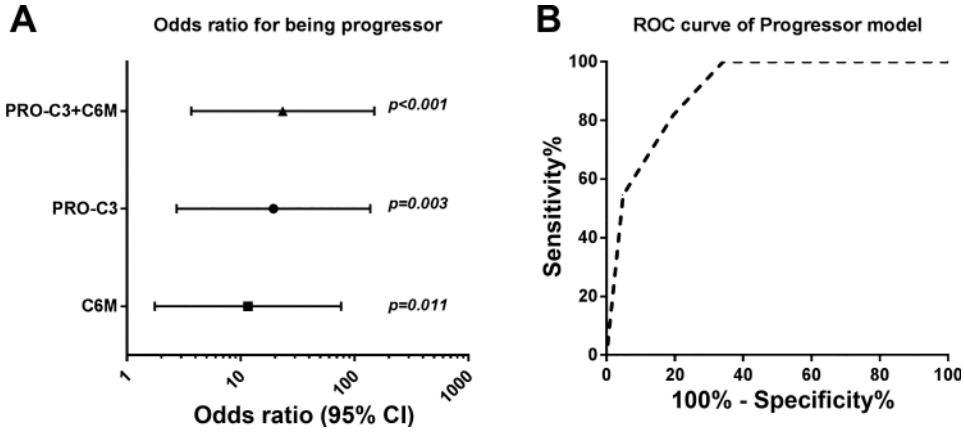

Fig. 2. Odds ratios (with 95% confidence intervals) for the progressor model including PRO-C3 and C6M (*A*) and the corresponding receiver operating characteristic (ROC) curve for detecting progressors ($n = 11$) from the rest of the population ($n = 41$) (*B*).

accurate identification of regressors, the associated criterion values of the ROC curve is listed in Table 4.

To test the robustness of the findings, we did internal cross-validation of the model using bootstrap resampling and LOOCV. This revealed a stable AUC of 0.78 for bootstrap resampling and 0.86 for bootstraping. However, LOOCV analysis showed a large drop in AUC to 0.57. The overall accuracy for PRO-C5 was 92.3%, i.e., 48 out of 52 patients were correctly classified.

DISCUSSION

In this study, we present data showing that, in our exploratory study, a collagen biomarker signature is potentially able to identify those patients with fibrotic liver disease that show fibrosis progression vs. those whose fibrosis regresses independently of treatment.

Collagens are the main constituents of the fibrotic extracellular matrix (ECM) (14) and represent the bulk of the unwanted structural proteins accumulating in fibrosis. Consequently, these collagens are a plausible basis for the development of noninvasive serological fibrosis biomarkers. Fibrillar collagens are synthesized with propeptides (33) that are proteolytically cleaved before the proteins can form collagen triple helices or other supramolecular structures in the ECM. These propeptides and even their subdomains that are generated by specific proteases can be quantified in serum or plasma to serve as surrogate biomarkers for ECM formation, turnover, or degradation (10, 19). First data suggest that the serum/plasma levels of certain procollagen fragments can be used to assess the balance of fibrogenesis or fibrolysis (9). In particular, three (pro)collagens have recently gained increased interest in assessing the dynamics of fibrosis, namely type III, V, and VI procollagens that have different physiological and pathophysiological functions and meanings. PRO-C3, a defined epitope of the NH_2-terminal propeptide of type III procollagen, is released by the protease ADAMTS-2 during collagen maturation, which is a prerequisite of efficient incorporation of collagen type III in collagen fibrils (26). In this line, PRO-C3 has been shown to diagnose and predict the progression of liver fibrosis and to identify fast progressors and responders to (antifibrotic) treatment in various etiologies of chronic liver disease, including nonalcoholic fatty liver disease (NAFLD), HCV, HBV, HIV/HCV coinfection, and alcoholic cirrhosis (5, 13, 15, 17, 24, 28). Consequently, not surprisingly, PRO-C3 was able to identify progressors in the current study.

Type VI collagen forms microfibrils that run in between interstitial collagen fibrils composed of collagens type I, III, and V. It is highly upregulated in the fibrotic space of Disse and portal tract stroma (8). Type VI procollagen has been identified as a "dangerous collagen" with important signaling functions related to the metabolic syndrome and fibrogenesis (12, 43). The C6M marker detects a fragment generated by matrix metalloproteinase (MMP)-2 and MMP-9 cleavage of type VI collagen, and serum levels are highly increased in liver fibrosis (48). In the current study, collagen type VI remodeling, as measured by C6M, was increased in patients who were progressing and qualified as a progression marker, and the combination with PRO-C3 may likely indicate accelerated turnover of interstitial collagen during fibrogenesis. Interestingly, the COOH-terminal propeptide of the α_3-chain of type VI procollagen (Endotrophin), a marker of type VI collagen formation, has recently been shown to be associated with adipose tissue inflammation and fibrosis, and the severity of the metabolic syndrome and insulin resistance in patients with type 2 diabetes and NAFLD (30, 31, 43). Endotrophin predicts the response of HbA_{1c} serum levels to insulin sensitizers (PPAR-γ

Table 3. *Correct identification of progressors using different cutoffs and their associated values from the ROC curve for the progressor model*

Cutoff	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
≥0.0192	100.0 (71.5–100.0)	0.00 (0.0–8.6)	21.2 (11.1–34.7)	
>0.0192	100.0 (71.5–100.0)	65.9 (49.4–79.9)	44.0 (24.4–65.1)	100.0 (87.2–100.0)
>0.1851	81.8 (48.2–97.7)	80.5 (65.1–91.2)	52.9 (27.8–77.0)	94.3 (80.8–99.3)
>0.2756	54.6 (23.4–83.3)	95.1 (83.5–99.4)	75.0 (34.9–96.8)	88.6 (75.4–96.2)
>0.8149	0.00 (0.0–28.5)	100.0 (91.4–100.0)		78.8 (65.3–88.9)

ROC, receiver operating characteristic; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

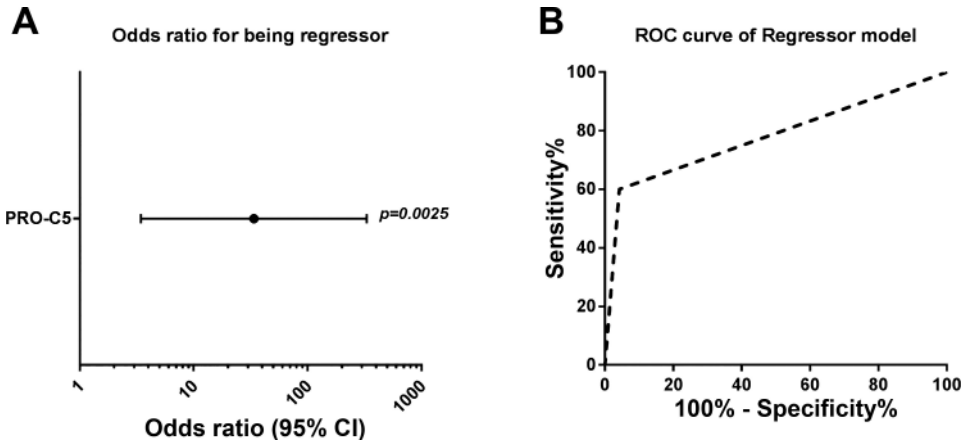

Fig. 3. Odds ratios (with 95% confidence intervals) for the regressor model including PRO-C5 (*A*) and the corresponding receiver operating characteristic (ROC) curve for detecting regressors ($n = 5$) from the rest of the population ($n = 47$) (*B*).

agonists) (12) and predicts the progression of kidney fibrosis (6). These characteristics indicate that type VI (pro)collagen is an ECM constituent with particular pathophysiological relevance, driving not only fibrotic remodeling but also metabolic derangement, a hallmark of NASH. Only insufficient amounts of samples were available for measurement of PRO-C6, which should be included in future studies to investigate whether endotrophin is a better predictor in a progression model and whether the model can be applied in other etiologies of chronic liver diseases, such as NASH.

Type V collagen is a fibrillar collagen involved in the assembly of tissue-specific collagen matrixes forming core fibrils on which collagen type I is deposited (3, 7, 39), hence regulating fibril formation and size (3, 20). High levels of PRO-C5 have already been shown to correlate with portal hypertension (16, 20) and to predict transplant-free survival in PSC patients (27). Interestingly, in the current study, low levels of PRO-C5, suggesting low levels of fibril formation, were associated with fibrosis reversal, which is in line with the current understanding of type V collagen formation and control of fibril structure.

Abnormal deposition of basement membrane material in advanced liver fibrosis and cirrhosis is mainly due to excessive synthesis of type IV collagen, and serum type IV collagen correlates with its hepatic tissue levels (46). In the current study, the two basement membrane remodeling markers, PRO-C4 and C4M, were not associated with either progression or regression of fibrosis, indicating that the turnover favors interstitial matrix remodeling rather than basement membrane remodeling in these patients.

In general, all fibrolysis markers, C3M, C4M, and C6M, failed to identify regressors of fibrosis. This may be because of the specifics of HCV-related fibrosis that may display a high basement membrane turnover (degradation and production) along with a continuously active HCV infection, as in the group studied that had at least Ishak stage 3–4 fibrosis and failed to respond to standard therapy. It could also be the result of the low number of patients in the regressor group or the duration of the study. The low number of patients is in general a limitation of the study, which may also explain the low specificity and positive predictive values for both the progressor and regressor models, despite the shown significances.

Recent studies of fibrosis evolution, based on sequential liver biopsies, demonstrated a sizable number of fibrosis regressors and progressors that could not be predicted by physiological parameters or noninvasive tools. This is particularly relevant for patients with NAFLD and NASH who can display very different degrees of fibrosis despite comparable liver enzyme values and risk factor profiles (41, 42). Thus, a natural history study from France described 20% of progressors and regressors in 70 patients over a mean follow-up of 3.7 yr (29), whereas a study from the United Kingdom found 42% progressors and 18% regressors in 108 patients with a follow-up of 6.6 yr (23). This highlights the need for identification of the patient segments for different purposes. Therefore, future studies and treatments will likely benefit from identification of both phenotypes and appropriate patient segmentation. An attractive solution to this problem is the development and validation of noninvasive diagnostic tools that will reliably measure the amount of scar tissue in the liver or preferably the dynamics of hepatic scar tissue formation (fibrogenesis), optimally combined with tools to measure scar tissue dissolution (fibrolysis) (37, 41). To support this aim, the Food and Drug Administration has developed the Accelerated Approval Pathway that allows drug developers to apply for drug approval with shorter studies based on validated and biologically plausible surrogate end points such as noninvasive biomarkers. This decision has reinforced drug and biomarker discoveries in liver fibrosis (36, 44). Importantly, liver fibrosis, especially when the result of NAFLD and NASH that affect a large part of most populations,

Table 4. *Correct identification of regressors using different cutoffs and their associated values from the ROC curve for the regressor model*

Cutoff	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
≥0.0426	100.0 (47.8–100.0)	0.0 (0.0–7.5)	9.6 (3.2–21.0)	
>0.0426	60.0 (14.7–94.7)	95.7 (85.5–99.5)	60.0 (14.7–94.7)	95.7 (85.5–99.5)
>0.6	0.0 (0.0–52.2)	100.0 (92.5–100.0)		90.4 (79.0–96.8)

ROC, receiver operating characteristic; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

with substantial numbers of regressors and progressors, in combination with the overall slow dynamics, poses a prime challenge for precision medicine, where preferably plasma biomarkers for patient stratification and assessment of fibrosis progression and regression are urgently needed.

A limitation to our study is that the sample size of included patients is relatively small after exclusion of the treatment arms. This makes statistical analysis challenging, which was especially reflected by the internal cross-validation for the regressor model. However, the data can be seen as valid proof of concept that a panel of specific collagen biomarkers can be used to identify progressors and regressors. The retrospective nature of this study can also be seen as a challenge to these calculations; samples have been stored in the freezer at −80° for up to 10 yr. However, the collagen markers measured in the study have been validated to remain stable in samples subject to long-term storage and several freeze-thaw cycles. A strength is that, in this study, we report the full data package of the markers that we have measured, including also those markers that did not possess predictive power in these patients. We were also not able to benchmark our markers against commonly used ECM biomarker tests such as the ELF test because of lack of sufficient serum. However, in contrast to other direct (ECM-derived) serum markers, our assays are directed toward well-defined epitopes that are unambiguously generated either during fibrogenesis or fibrolysis.

Conclusion. We identified three out of six selected plasma collagen "fingerprint" markers that are associated with fibrosis progression and regression. This marker panel will be further validated in ongoing larger prospective studies of patients with chronic liver diseases, especially NAFLD, who will be followed with or without therapeutic intervention.

GRANTS

The project was supported by "Den Danske Forskningsfond" and is part of the European Union-funded European Project on Steatohepatitis, and Liver Investigation: Testing Marker Utility in Steatohepatitis, which has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under Grant Agreement No. 777377. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and European Federation of Pharmaceutical Industries and Associations.

DISCLOSURES

M.A.K. is an employee of and owns stocks/stock options in Nordic Bioscience. M.J.U., D.J.L., D.G.R., S.H.N., and S.T.H. are employees of Nordic Bioscience. D.S., Z.G., and K.P. declare no conflicts of interest.

M.A.K., M.J.U., and D.J.L. are the original inventors and developers of the PRO-C3 assay. A patent application has been submitted on this assay.

AUTHOR CONTRIBUTIONS

M.A.K. conceived and designed research; M.A.K., S.T.H., and D.G.K.R. analyzed data; M.A.K., Y.L., D.G.K.R., S.H.N., D.J.L., Z.G., R.H.A., K.P., and D.S. interpreted results of experiments; M.A.K. and S.T.H. drafted manuscript; M.A.K., S.T.H., Y.L., D.G.K.R., M.J.N., S.H.N., D.J.L., Z.G., R.H.A., K.P., and D.S. edited and revised manuscript; M.A.K., S.T.H., Y.L., D.G.K.R., M.J.N., S.H.N., D.J.L., Z.G., R.H.A., K.P., and D.S. approved final version of manuscript; S.T.H. and D.G.K.R. prepared figures.

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