Use of Plasma Fragments of Propeptides of Type III, V, and VI Procollagen for the Detection of Liver Fibrosis in Type 2 Diabetes

Fernando Bril,[1,2] Diana Julie Leeming,[3] Morten Asser Karsdal,[3] Srilaxmi Kalavalapalli,[1] Diana Barb,[1] Jinping Lai,[4] Matthew Rabe,[1] and Kenneth Cusi[1,2]

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OBJECTIVE

This study assessed the utility of plasma fragments of propeptides of type III (PRO-C3), V (PRO-C5), and VI (PRO-C6) procollagen for the detection of liver fibrosis in patients with type 2 diabetes mellitus (T2DM).

RESEARCH DESIGN AND METHODS

Patients with T2DM ($n = 191$) underwent an oral glucose tolerance test, a liver ^{1}H-MRS, and a liver biopsy when indicated. PRO-C3, PRO-C5, and PRO-C6 were blindly assessed.

RESULTS

PRO-C3 performed well for the diagnosis of moderate-to-advanced (area under the receiver operating characteristic curve [AUROC] 0.81 [95% CI 0.74–0.88]) and advanced (AUROC 0.88 [0.80–0.95]) fibrosis in T2DM patients. Its performance was similar to that of AST to platelet ratio index (APRI) (AUROC 0.83 and 0.87, respectively) and Fibrosis-4 (FIB-4) (AUROCs 0.83 and 0.86, respectively) scores. Use of PRO-C5 and PRO-C6 did not improve the accuracy to detect liver fibrosis. After 18 months, PRO-C3 changes were associated with changes in fibrosis stages.

CONCLUSIONS

PRO-C3 performed well for the detection of fibrosis in T2DM patients and showed promising results for prediction of histological changes in fibrosis stage with treatment.

Patients with type 2 diabetes mellitus (T2DM) are at increased risk of developing nonalcoholic steatohepatitis (NASH) (1). Several noninvasive clinical panels, combining plasma biomarkers and anthropometric measurements, have been developed to predict the presence of NASH or advanced fibrosis (2–4). However, their specific performance in T2DM patients is frequently not assessed, and results are often extrapolated from studies in patients without diabetes (1). Only recently have efforts focused in a population exclusively of patients with T2DM, but results have been mixed and not widely embraced by health care providers (5,6).

Fragments of the propeptide of type III, V, and VI procollagen (PRO-C3, PRO-C5, and PROC6), which are generated from procollagen cleavage during liver collagen deposition, have recently been used to predict fibrosis in patients with chronic liver diseases, such as hepatitis C (7–11). The aim of the current study was to assess their utility for the diagnosis of nonalcoholic fatty liver disease (NAFLD)-induced moderate-to-advanced (stages 2–4) and advanced (stages 3–4) fibrosis in a large

[1]Division of Endocrinology, Diabetes and Metabolism, University of Florida, Gainesville, FL
[2]Malcom Randall Veterans Administration Medical Center, Gainesville, FL
[3]Nordic Bioscience A/S, Herlev, Denmark
[4]Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL

Corresponding author: Kenneth Cusi, kenneth.cusi@medicine.ufl.edu

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cohort of T2DM patients. Moreover, their use to predict changes in fibrosis stage after 18 months of follow-up was also assessed.

RESEARCH DESIGN AND METHODS

Subjects

Patients with T2DM were recruited from the general population, and from hepatology/endocrinology clinics, in Gainesville, FL, and San Antonio, TX. Some of these patients were previously included in other studies assessing other clinical panels (12). Only stable doses of metformin, sulfonylureas, and/or insulin were allowed as glucose-lowering medications. Exclusion criteria included significant alcohol consumption (≥30 g/day for males and ≥20 g/day for females), any liver disease other than NASH, and medications known to affect NAFLD. A subgroup of patients were followed for 18 months as part of randomized controlled trials. Details and results from those randomized controlled trials have previously been published (13,14). The study was approved by both institutional review boards, and written informed consent was obtained from each patient prior to participation.

Study Design

All patients underwent proton MRS (^{1}H-MRS) to measure intrahepatic triglyceride content as previously reported (15), a 2-h OGTT (with blood collection every 30 min), and a percutaneous liver biopsy if patients had NAFLD. Baseline plasma samples ($n = 191$) and available month-18 samples ($n = 79$) were blindly provided to Nordic Bioscience (Herlev, Denmark). Plasma PRO-C3, PRO-C5, and PRO-C6 were analyzed using competitive ELISAs as previously described (9).

Statistical Analysis

Data was presented as mean ± SD unless otherwise specified. Categorical variables were compared performing χ^2 or Fisher exact test. Kruskal-Wallis or ANOVA were used for numeric variables. Hadi's method was used to assess for outliers based on PRO-C3 concentration measurements, and outliers ($n = 3$, all at month 18) were removed from the analysis. Multiple logistic regression analysis for the prediction of moderate-advanced (defined as fibrosis stages 2–4) and advanced (defined as fibrosis stages 3–4) fibrosis were performed by forward selection of variables with $P < 0.20$ in the univariate analyses. Analyses were performed with Stata 11.1 (StataCorp LP, College Station, TX).

RESULTS

Baseline Characteristics

Mean age was 59 ± 8 years, and 86% were male, with a predominance of Caucasians (67%), followed by Hispanics (21%) and African Americans (12%). Mean BMI was 33.5 ± 4.7 kg/m^2, HbA_{1c} 7.2 ± 1.2%, and HOMA of insulin resistance (HOMA-IR) 5.5 ± 5.0. BMI and intrahepatic fat were lower in patients without NAFLD but similar among patients with NAFLD, independently of their fibrosis stage. Patients with more severe fibrosis showed higher insulin levels, higher aminotransferases, and worse liver inflammation and ballooning in histology (Supplementary Table 1). Patients with different fibrosis stages had similar age, sex, ethnicity, diabetes control, or glucose-lowering drug use. Patients without NAFLD by ^{1}H-MRS were considered as not having fibrosis. Of note, sensitivity analyses excluding these patients showed no significant changes.

Diagnosis of Liver Fibrosis

Plasma PRO-C3 showed higher levels with worsening fibrosis stages, with the most significant increase at stages 3/4 (stage 0 = 8.8 ± 3.2 vs. stage 1 = 10.6 ± 4.6 vs. stage 2 = 12.7 ± 4.6 vs. stages 3/4 = 23.5 ± 15.9 ng/mL, $P < 0.001$) (Supplementary Fig. 1). Plasma PRO-C5 and PRO-C6 did not show any significant variation with increasing fibrosis stage.

PRO-C3 levels performed well as a predictive tool for moderate-advanced (area under the receiver operating characteristic curve [AUROC] 0.81 [0.74–0.88]) and

Table 1—Performance of plasma PRO-C3 as a single marker or composite score for the diagnosis of moderate-advanced and advanced fibrosis compared with indirect composite scores

	AUROC (95% CI)	*P* value of AUROC compared with PRO-C3	Optimum cutoff point	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Diagnosis of moderate-to-advanced fibrosis (stages 2, 3, or 4)							
Plasma PRO-C3	0.81 (0.74–0.88)	—	9.70 ng/mL	86 (74–94)	68 (59–75)	50 (39–61)	93 (86–97)
APRI	0.83 (0.76–0.90)	0.73	0.46	72 (58–84)	86 (79–91)	66 (51–78)	89 (83–94)
FIB-4	0.73 (0.65–0.81)	0.10	1.30	70 (55–82)	87 (81–92)	67 (53–80)	89 (82–94)
ADAPT	0.77 (0.69–0.84)	0.06	6.20	72 (58–84)	71 (63–79)	49 (37–60)	87 (80–93)
Plasma PRO-C3 plus clinical parameters#	0.87 (0.81–0.94)	0.10	−0.40	71 (57–83)	94 (88–97)	81 (67–92)	90 (83–94)
Diagnosis of advanced fibrosis (stages 3 or 4)							
Plasma PRO-C3	0.88 (0.80–0.95)	—	13.2 ng/mL	82 (62–94)	82 (76–88)	43 (29–58)	96 (92–99)
APRI	0.87 (0.81–0.93)	0.98	0.42	89 (71–98)	75 (67–81)	38 (26–50)	98 (93–100)
FIB-4	0.83 (0.75–0.92)	0.43	1.76	74 (54–89)	79 (72–85)	38 (25–52)	95 (89–98)
ADAPT	0.86 (0.77–0.94)	0.56	6.58	82 (62–94)	81 (74–87)	42 (29–57)	96 (92–99)
Plasma PRO-C3 plus clinical parameters*	0.91 (0.86–0.96)	0.23	−1.40	82 (62–94)	85 (78–90)	50 (35–65)	96 (91–99)

#Only AST, HOMA-IR (fasting glucose [in mg/dL] * fasting insulin [in μU/mL]/405), sex, and weight remained independently associated with moderate-advanced fibrosis in the multiple logistic regression model (model: 0.11 × PRO-C3 + 0.05 × AST + 0.17 × HOMA-IR + 2.16 × sex [1 = male; 0 = female] − 0.03 × weight [in kg] − 4.021109; pseudo-R^2 = 0.39). *Only AST, HOMA-IR, and platelets remained independently associated with advanced fibrosis in the multiple logistic regression model (model: 0.14 × PRO-C3 + 0.03 × AST + 0.14 × HOMA-IR − 0.01 × platelets − 2.807115; pseudo-R^2 = 0.41).

advanced (AUROC 0.88 [0.80–0.95]) fibrosis among patients with T2DM (Table 1 and Supplementary Fig. 2). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the optimum cutoff points of 9.7 ng/mL for moderate-advanced fibrosis (prevalence 27%) and 13.2 ng/mL for advanced fibrosis (prevalence 14%) can be found in Table 1. If considering an indeterminate ("grey") zone between 9.7 and 13.2 ng/mL, only a small proportion of patients (n = 39 [20.4%]) would be classified as "indeterminate," and the performance of PRO-C3 would improve to the following: sensitivity = 82% and 96%, specificity = 83% and 78%, PPV = 63% and 43%, and NPV = 93% and 99% for moderate-advanced and advanced fibrosis, respectively. This implies that 83% and 80% of patients would be correctly classified for moderate-advanced and advanced fibrosis, respectively.

Comparison of PRO-C3 Against Other Clinical Scores

In Table 1, the performance of plasma PRO-C3, alone or combined with clinical parameters, was compared with other simple clinical panels based on routine laboratory results and indirect composite scores of liver fibrosis (i.e., AST to platelet ratio index [APRI], Fibrosis-4 [FIB-4]), as well as the already reported score based on Age, presence of DiAbetes, PRO-C, and plaTelet count, known as ADAPT (6). All methodologies/scores provided an acceptable discrimination for moderate-advanced fibrosis (all AUROCs >0.80) and even better for advanced fibrosis (all AUROCs >0.85). No significant differences were observed when AUROCs were compared with each other.

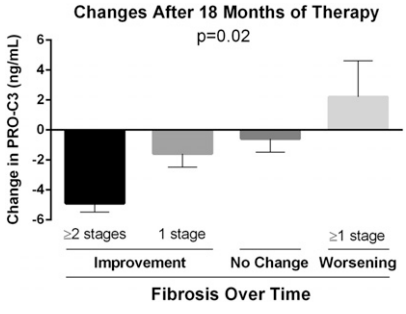

Figure 1—Relationship between PRO-C3 and histological change in fibrosis stage over time.

Longitudinal Use of Plasma PRO-C3

Patients were followed for 18 months after treatment with placebo (n = 26), vitamin E (n = 24), pioglitazone (n = 4), or combination of vitamin E and pioglitazone (n = 22). Changes in plasma PRO-C3 were significantly correlated with changes in fibrosis stage (r = 0.34, P = 0.002) (Fig. 1). Moreover, changes in plasma PRO-C3 were able to identify patients with ≥2-point improvement in fibrosis (area under the curve 0.85 [95% CI 0.77–0.94]), but not 1-point improvement (area under the curve 0.67 [0.54–0.79]). Changes in PRO-C5 and PRO-C6 were not significantly associated with changes in fibrosis over time.

CONCLUSIONS

Results from the current study suggest that the use of PRO-C3 in patients with T2DM can appropriately identify subjects with moderate-advanced and advanced fibrosis. Moreover, measuring PRO-C3 levels over time may aid in predicting changes in fibrosis stages over time, making it a potential pharmacodynamic marker for following patients with NASH. However, while promising, due to the significant overlap among these relatively small groups, this test cannot yet be recommended to replace a liver biopsy in assessment of NASH fibrosis. Larger prospective studies are needed to fully assess the true value of PRO-C3 as a potential fibrosis biomarker to assess the natural history of the disease or response to treatment.

Prior evidence of the utility of plasma PRO-C3 to detect liver fibrosis came from cohorts of patients with hepatitis B and C (10,16). More recently, PRO-C3 has been combined with simple clinical parameters (age, presence of diabetes, and platelets) to identify advanced fibrosis in patients with NAFLD in a model called ADAPT (6). However, the ADAPT model is strongly based on the presence of diabetes to detect those patients with advanced fibrosis. Whether this model would hold when facing a cohort of only patients with T2DM (as seen in endocrinology clinics) was unknown. In our cohort of patients with T2DM, the ADAPT model performed nonsignificantly worse than PRO-C3 alone for the diagnosis of moderate-advanced and advanced fibrosis. Another key question has been whether PRO-C3 can perform significantly better than "old and simple" clinical scores, such as APRI and FIB-4. Of note, we observed no significant differences between the performances of plasma PRO-C3, APRI, and FIB-4. Therefore, while PRO-C3 appears as a reliable stand-alone tool to identify patients with advanced fibrosis, simple panels relying on plasma aminotransferases and platelets may perform just as well.

In summary, PRO-C3 was useful to distinguish patients with moderate-advanced and advanced fibrosis in a large cohort of patients with T2DM. This is important, as patients with T2DM and NAFLD tend to behave differently than patients without T2DM, with many diagnostic tools underperforming in this population (12). When compared with simple indirect fibrosis panels based on routine laboratories, PRO-C3 performed similarly, suggesting that any of these options are valid for noninvasive measurements of fibrosis risk and should be considered by the clinician on a careful case-by-case analysis. While a liver biopsy remains the gold standard for the diagnosis of NASH, future studies will help define the role of PRO-C3, and its combination with imaging and other diagnostic algorithms, for the noninvasive diagnosis of NASH.

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Author Contributions. F.B. contributed to patient recruitment and follow-up, data acquisition and interpretation, statistical analysis, and writing and editing of the manuscript. D.J.L. and M.A.K. performed the PRO-C3 analysis, aided in discussion of data analysis, provided input, and approved the manuscript. S.K., D.B., and M.R. contributed to data acquisition and critical revision of the manuscript. J.L. contributed to reading of liver biopsies and critical revision of the manuscript. K.C. contributed to study design and funding, patient recruitment and follow-up, data acquisition and interpretation, and critical revision and editing of the manuscript. F.B. and K.C. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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