PRO-C3 (A COLLAGEN NEOEPITOPE PROPEPTIDE) IDENTIFIES PATIENTS WITH PROGRESSIVE LIVER FIBROSIS AND RESPONDERS TO ANTI-FIBROTIC THERAPY

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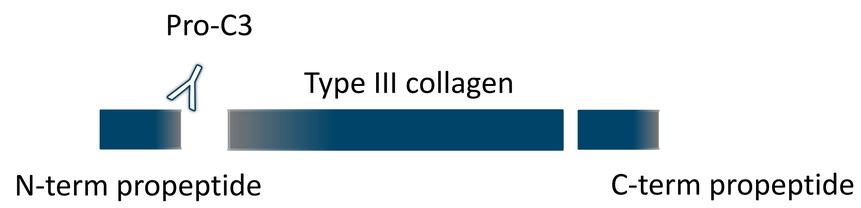
BACKGROUND: There are no approved treatments for liver fibrosis. To aid development of anti-fibrotic therapies, biomarkers that can identify patients with progressive fibrosis and permit monitoring of the response to anti-fibrotic therapy are much needed.
The aim was to validated Pro-C3, a novel neoepitope specific serum marker for collagen type III deposition, as a biomarker to identify responders to anti-fibrotic therapies, and as an early marker to assess efficacy of anti-fibrotic therapy.

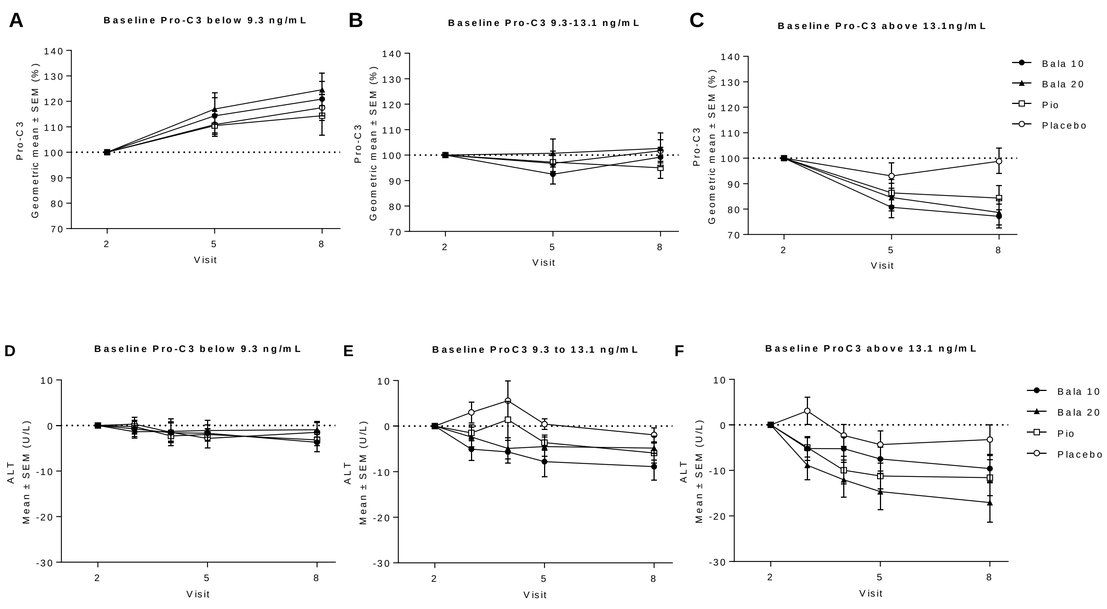
METHODS: Data were generated for 299 individuals from a phase III study of balaglitazone in late stage type 2 diabetics (BALLET trial; NCT00515632), and 198 individuals from a phase II, randomized, double-blinded, placebo-controlled multicenter study to determine the anti-fibrotic effect of farglitazar (NCT00244751) in patients with advanced hepatitis C. The latter included matched follow-up liver biopsies, and other disease relevant parameters. Pro-C3 was assessed in serum samples using a competitive ELISA. Fatty liver index (FLI: BMI, waist circumference, triglycerides and GGT) was used to support the diagnosis of fatty liver in the BALLET patients. A FLI < 30 rules out fatty liver, while FLI ≥ 60 rules in fatty liver.

Pro-C3
Released N-terminal
pro-peptide of type III collagen



BALLET trial:

	Pro-C3+, FLI>60 N=94	Pro-C3-, FLI<60 N=205	P-VALUE
TREATMENT	Bala 10 mg: n=25 Bala 20 mg: n=21 Pio 45 mg: n=27 Placebo n= 21	Bala 10 mg: n=48 Bala 20 mg: n=48 Pio 45 mg: n=57 Placebo n= 52	-
AGE (YRS)	60.9 (7.9)	60.4 (8.7)	0.66
GENDER	Female: 35 (37%) Male: 59 (63%)	Female: 63 (31%) Male: 142 (69%)	0.29
BMI (KG/M²)	35.7 (5.9)	32.5 (4.5)	<0.0001
WAIST CIRCUMFERENCE (CM)	119 (14)	111 (11)	<0.0001
HIP CIRCUMFERENCE (CM)	116 (12)	110 (9)	<0.0001
DXA TOTAL BODY FAT MASS (KG)	37.7 (9.8)	31.7 (8.4)	<0.0001
DXA TRUNK FAT MASS (KG)	22.6 (5.2)	18.8 (5.1)	<0.0001
BLOOD HBA1C (%)	8.7 (1.4)	8.6 (1.4)	0.59
SERUM GLUCOSE (MMOL/L)	9.6 (3.1)	9.4 (3.4)	0.78
SERUM ALT (U/L)	41 (21)	29 (13)	<0.0001
SERUM AST (U/L)	38 (16)	28 (8)	<0.0001
SERUM GGT (U/L)	69 (58)	43 (39)	<0.0001
SERUM ALP (U/L)	184 (55)	170 (49)	0.02
SERUM BILIRUBIN (MMOL/L)	8 (3.5)	9 (4.4)	0.24
SERUM TRIGLYCERIDES (MMOL/L)	1.94 (1.08)	1.74 (1.08)	0.15
SERUM CHOLESTEROL (MMOL/L))	4.35 (0.85)	4.36 (1.00)	0.91
SERUM HDL CHOL (MMOL/L)	1.21 (0.24)	1.29 (0.33)	0.02
SERUM LDL CHOL (MMOL/L)	2.58 (0.81)	2.58 (0.91)	0.99



High levels of Pro-C3 are useful for identification of responders to the putative anti-fibrotic effect of the PPARγ agonist. Changes in Pro-C3 as a function of agonists or placebo A) in the lowest (<9.3 ng/mL), B) in the middle (9.3-13.1 ng/mL), and C) in the highest (>13.1 ng/mL) tertile. Changes in ALT levels as a function of agonists or placebo in the D) lowest (<9.3 ng/mL), E) in the middle (9.3-13.1 ng/mL), F) in the highest (>13.1 ng/mL) tertile.

Farglitazar trial:

Ishak stage	2 (n=78)	3 (n=88)	4 (n=28)	P-value
Age, years	52 [50.3-53.2]	52 [50.4-53.2]	52 [49.6-53.8]	0.828
BMI, kg/m²	28.3 [27.3-29.4]	29.1 [28.0-30.1]	28.6 [26.8-30.3]	0.702
Gender (Male/Female)	53 (68%) / 25 (32%)	54 (61%) / 34 (39%)	18 (64%) / 10 (36%)	
HAI	6.2 [5.9-6.5]	6.6 [6.2-6.9]	7.5 [6.8-8.2]	0.002
Steatosis grade	1.1 [1.0-1.3]	1.3 [1.1-1.4]	1.3 [1.0-1.7]	0.456
Collagen	0.05 [0.04-0.07]	0.08 [0.06-0.09]	0.10 [0.06-0.13]	0.001
ALT, IU/L	58.9 [49.6-68.3]	77.2 [64.1-90.4]	104.6 [74.6-134.7]	0.004
AST, IU/L	59.7 [50.9-68.5]	57.8 [51.7-63.9]	97.9 [76.0-119.9]	<0.001
FibroTest, unit	0.6 [0.5-0.6]	0.6 [0.6-0.7]	0.7 [0.7-0.8]	0.012
C3M, ng/mL	17.6 [16.5-18.7]	18.8 [17.4-20.2]	20.8 [17.6-23.9]	0.126
Pro-C3, ng/mL	17.3 [15.4-19.1]	18.6 [17.1-20.4]	31.2 [21.7-40.6]	<0.001

A) Baseline Pro-C3 level in CHC patients stratified according to changes in Ishak stage after 52 weeks. Group -1: decrease of 1 in Ishak stage; Group 0: no change in Ishak stage; Group 1: increase of 1 in Ishak stage; and Group 2: increase of 2 Ishak stages. The dotted horizontal lines in A) represent the biomarker levels of a normal healthy population. Number of patients in each group is shown in each panel. B-D: High levels of Pro-C3 identify responders to anti-fibrotic activity of farglitazar. Patients with chronic hepatitis C and intermediate fibrosis (Ishak stage 2-4) that did not respond to prior antiviral therapy. B) Changes in Pro-C3 levels as a function of time and therapy in individuals with low (left) vs high (right) Pro-C3 baseline levels. P-values reflect differences in LS means between placebo and treatment with farglitazar. The p-value in parenthesis reflects a direct comparison between the endpoint values.C-D) Changes in Ishak stage as a function of time and therapy in individuals with low (C) vs high (D) Pro-C3 baseline levels. Number of patients in the Low Pro-C3 group: Placebo week 0: n=44; week 52 n=32; Treatment week 0: n=86; week 52 n=70. Number of patients in the High Pro-C3 group: Placebo week 0: n=25; week 52: n=20; Treatment week 0: n=45; week 52: n=36.

CONCLUSIONS:
Elevated Pro-C3 levels were highly indicative of active fibrogenesis and was identify patients most likely to benefit from anti-fibrotic treatment and monitoring of treatment effects. Serum Pro-C3 facilitates patient selection and should help to speed up anti-fibrotic drug development and validation.

Conflicts of interests:
Karsdal, Nielsen and Leeming are full-time employees at Nordic Bioscience. Karsdal holds stocks in Nordic Bioscience.
All other authors has no conflicts of interests.

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