Plasma collagen type III (Pro-C3) levels associate with severity of histological features of non-alcoholic steatohepatitis and fibrosis within the screening population from the CENTAUR study

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Disclosures

- Diana J. Leeming and Morten A. Karsdal are full-time employees and stock owners of Nordic Bioscience A/S
- Mette J. Nielsen is a full-time employee of Nordic Bioscience A/S
- Zachary Goodman has received research support from Alexion, Allergan plc, Cempra, Conatus, Galectin, Gilead, Intercept, and Tobira Therapeutics, Inc.
- Eric Lefebvre, Star Seyedkazemi, Brian Wiens, Laurent Fischer, and Pamela Vig are employees of Allergan plc
- Scott Friedman has received consultancy fees from Allergan plc
- Vlad Ratziu has received consultancy fees from Abbott, Allergan plc, AstraZeneca, Boehringer Ingelheim, Galmed, Genfit, Intercept, Isis, Novartis, Pfizer, Tobira Therapeutics, Inc., and Zafgen

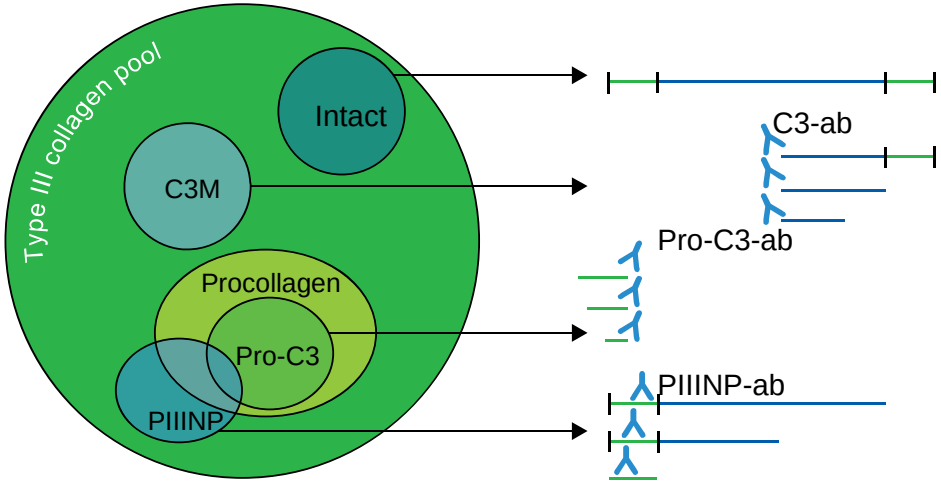
Introduction

- Non-alcoholic fatty liver disease (NAFLD) is prevalent in approximately 25% of the global population
  - Approximately 10–20% of patients with NAFLD have non-alcoholic steatohepatitis (NASH), an advanced form defined by steatosis, hepatocellular ballooning, and lobular inflammation[1]
- NASH is a fibrogenic phenotype of NAFLD that may progress to cirrhosis and liver failure
- There is a need for non-invasive tests to detect fibrosis amongst individuals at high risk for developing NASH

1. World Gastroenterology Organisation Global Guidelines. 2012. Available at: http://www.worldgastroenterology.org/UserFiles/file/guidelines/nafld-nash-english-2012.pdf

Introduction and objective

- Type III collagen synthesis is upregulated in liver fibrosis
- Pro-C3 is a neo-epitope marker reflecting true type III collagen formation
- Pro-C3 is diagnostic as well as able to predict progression of liver fibrosis in hepatitis C in patients at baseline over a 1-year period[1,2]
- Pro-C3 is a candidate marker for identifying a progressing subpopulation of patients with NAFLD[a]



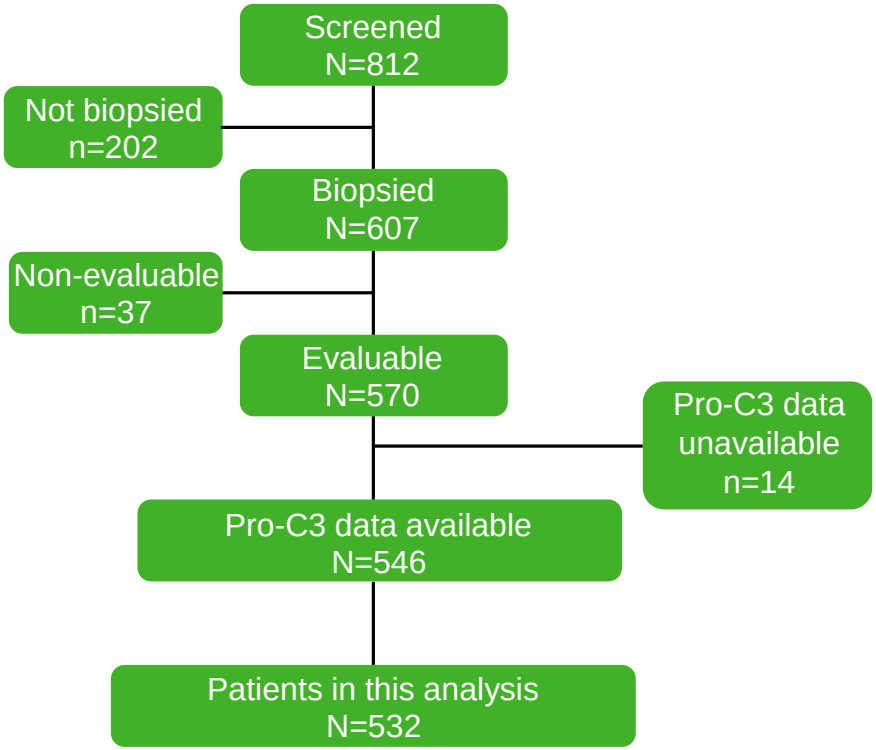
Objective: To investigate associations between Pro-C3 and histologic features of NASH and fibrosis in patients screened for the CENTAUR study

[a]Predefined cut-off points for evaluation of the diagnostic value of Pro-C3 were defined by the optimal diagnostic ability of Pro-C3[2] and cut-off for being a fast progressor in two different cohorts of patients with hepatitis C[1]
ab, antibody; C3M, type III collagen neo-epitope fragment; PIIINP, type III collagen procollagen peptide

1. Nielsen MJ et al. Liver Int 2015;35:429–37
2. Nielsen MJ et al. PLoS One 2015;10:e0137302

Methods: Sample collection

- CENTAUR is a 2-year, Phase 2b, randomized, double-blind, placebo-controlled clinical trial of cenicriviroc (CVC) to assess the efficacy and safety for the treatment of NASH in adults with liver fibrosis[1]
- In this exploratory analysis using samples from the CENTAUR study, 532 patients with evaluable biopsies and available Pro-C3 data were analyzed from the 812 screened



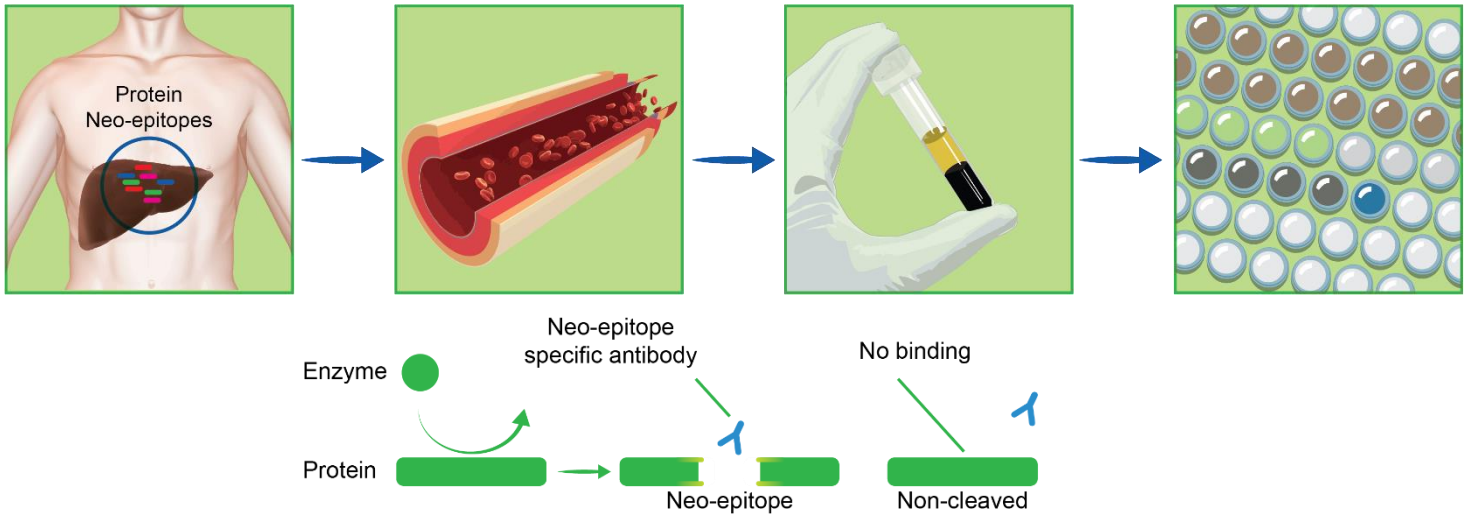
Key eligibility criteria	
	• A biopsy diagnosis of NASH, NAS ≥4, and liver fibrosis stage 1–3 (NASH CRN) • Documented type 2 diabetes, high BMI (25 kg/m^2) with ≥1 criteria of metabolic syndrome, or bridging fibrosis and/or NAS ≥5

BMI, body mass index; NAS, NAFLD activity score;
NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network

1. Friedman S et al. Contemp Clin Trials 2016;47:356–65 (NCT02217475)

Methods: Pro-C3 assessment and correlation with liver histology

- Pro-C3 was assessed in EDTA plasma using a competitive enzyme-linked immunosorbent assay (ELISA, Nordic Bioscience A/S, Denmark), employing the protein fingerprint technology[1]



- Pro-C3 was correlated with liver histology based on the NASH CRN scoring system

Fibrosis stage subgroups	n
F0–1	267
F0–2	155
F2–4	267
F3–4	377

EDTA, ethylenediaminetetraacetic acid

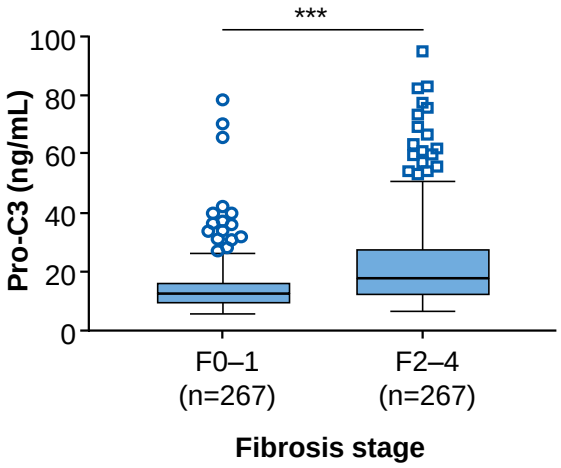
1. Nielsen MJ et al. Am J Transl Res 2013;5:303–15

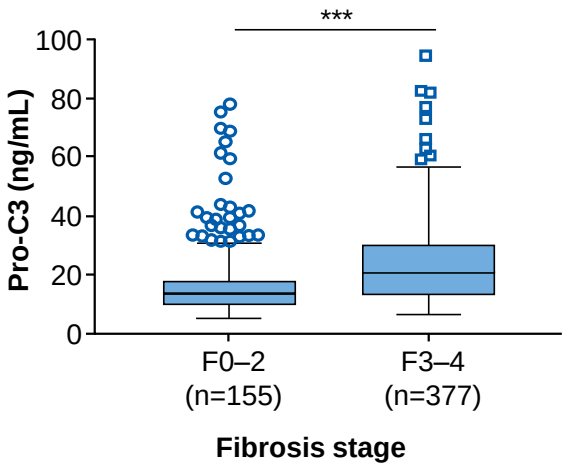
Demographics and disease characteristics

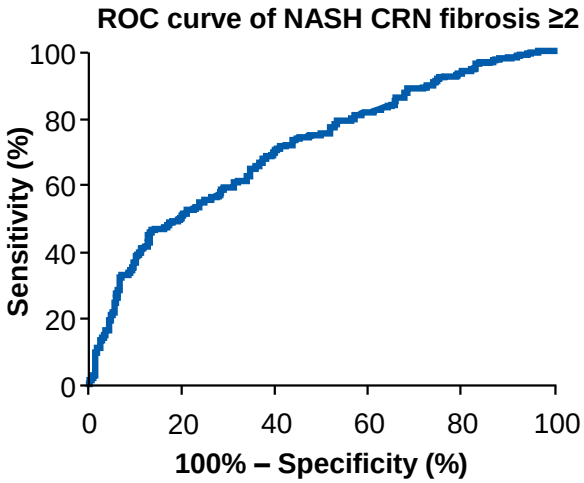
Category	n[a]	Value
Mean age, years (SD)	532	53.3 (11.6)
Male, n (%)	532	271 (51)
Mean BMI, kg/m^2 (SD)[b]	279[b]	34.0 (6.5)
Documented type 2 diabetes, n yes / n no (%)	532	216 / 316 (41)
Mean HbA1c, % (SD)[b]	278[b]	6.5 (1.3)
Mean ALT / AST, U/L (SD)	532 / 532	58.0 (37.9) / 41.3 (23.4)
Mean alkaline phosphatase, U/L (SD)	532	80.0 (24.2)
NAS ≥5, n (%)	532	259 (49)
NASH CRN fibrosis stage, n (%) ≤2 >2	532	 377 (71) 155 (29)

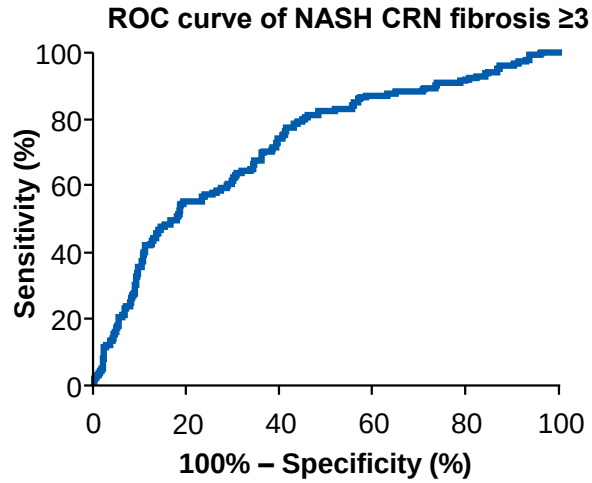
[a]532 samples were screened in total; [b]Values were not available in all screened patients
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HbA1c, hemoglobin A1c; SD, standard deviation

Pro-C3 distinguishes between fibrosis stages





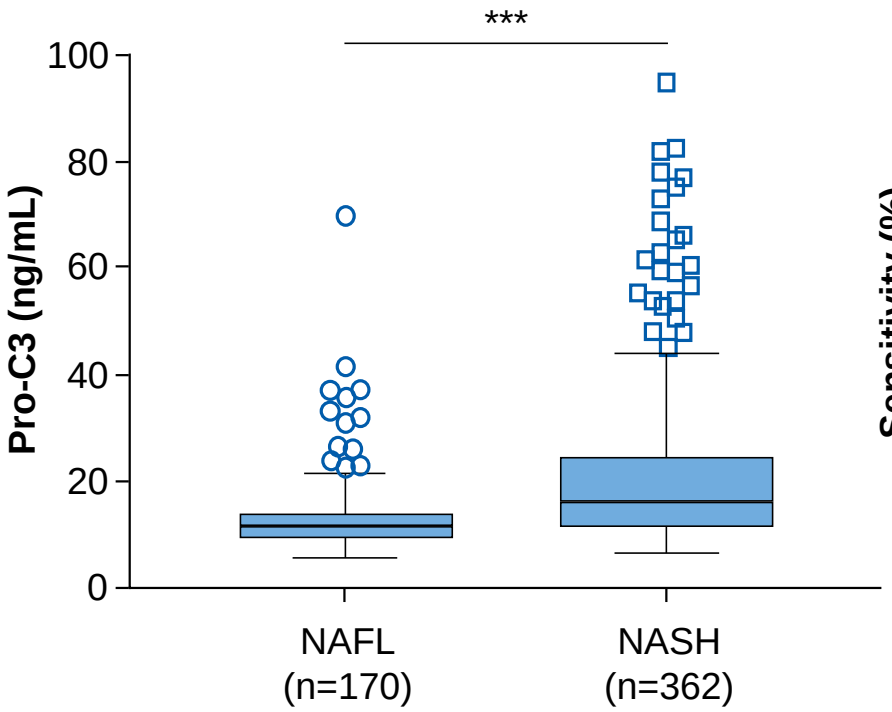


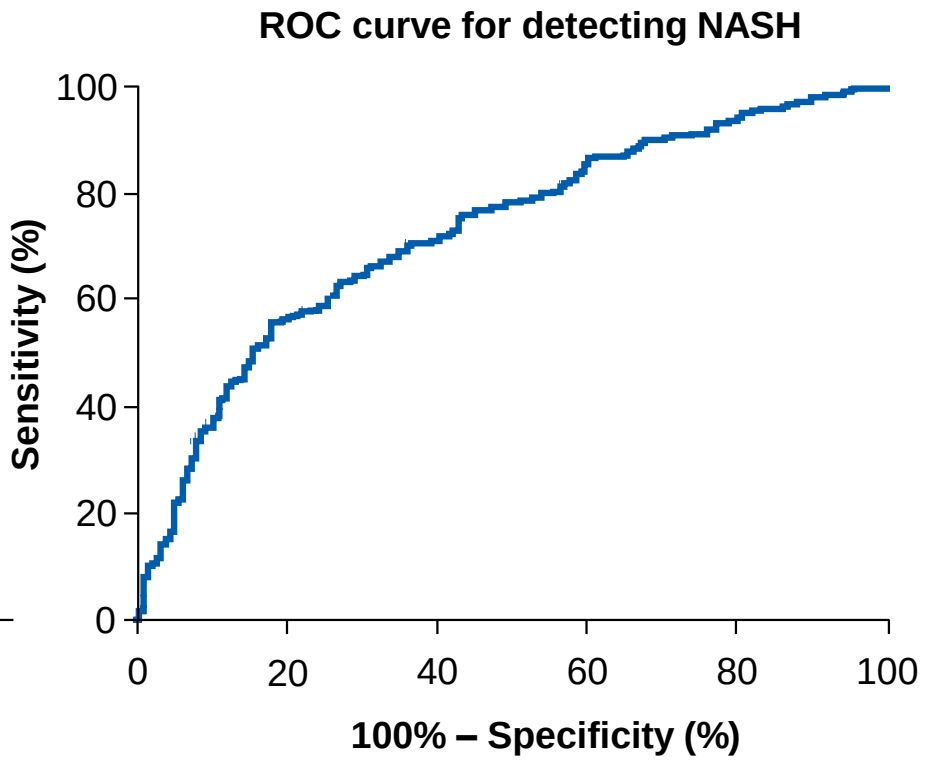


	Pro-C3			
Stage	F0–1 vs. F2–4		F0–2 vs. F3–4	
N	267 vs. 265		155 vs. 377	
Prev %	50.2		29.1	
AUC	0.71		0.726	
SD	0.022		0.025	
P value	<0.0001		<0.0001	
Cut-off	>20.2[a]	>22.2[b]	>21.3[a]	>25.3[b]
Sensitivity	44.6	38.2	49	37.4
Specificity	87.2	90.2	83.8	89.7
PPV	77.8	79.7	55.4	59.8
NPV	61	59.1	80	77.7

Box-and-whisker plots are shown as Tukey box plots; [a]Cut-off generated from Farglitazar cohort (HCV): Nielsen MJ et al. Liver Int 2015;35:429–37; [b]Cut-off generated from an Australian cohort (HCV): Nielsen MJ et al. PLoS One 2015;10:e0137302
AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic

Pro-C3 distinguishes NAFL from NASH





Marker	Stage	N	Prev %	AUC	SD	P value	Cut-off	Sensitivity	Specificity	PPV	NPV
Pro-C3	NAFL vs. NASH	170 vs. 362	68.0	0.732	0.023	<0.0001	>14.7[a] >20.2[b] >21.2[c]	55.8 37.6 34.8	82.4 90.0 91.8	87.1 88.9 90.0	46.7 40.4 39.8

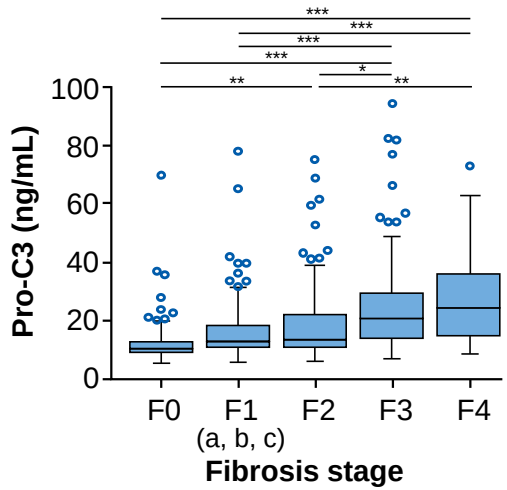
Box-and-whisker plot is shown as a Tukey box plot; [a]Computer generated cut-off based on highest sensitivity and specificity in this cohort; [b]Cut-off generated from Farglitazar cohort (HCV); [c]Cut-off generated separating progressors from stable patients in Farglitazar cohort

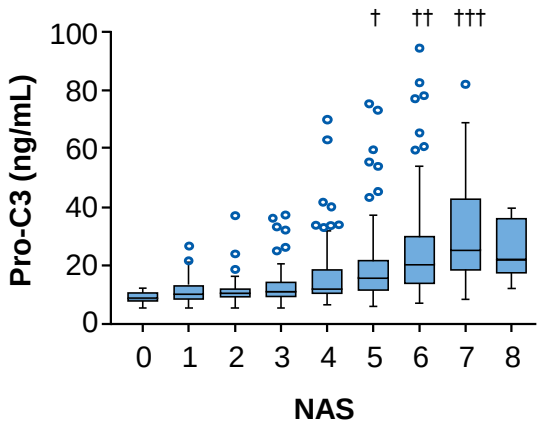
AST and ALT are significantly elevated in the high Pro-C3 groups

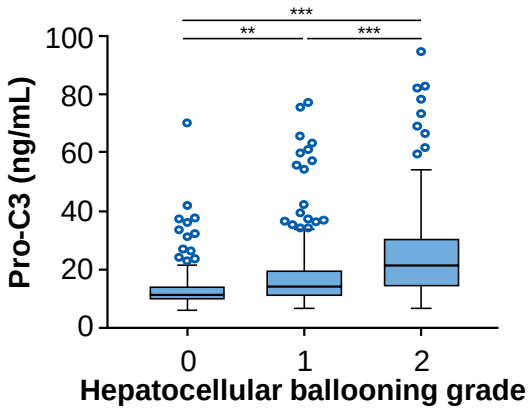
Category	Pro-C3 <20.2 ng/mL		Pro-C3 ≥20.2 ng/mL		P value
Male, n (%)	201 (53)		70 (46)		0.1066
Documented type 2 diabetes, n (%)	149 (39)		67 (44)		0.3843
	n[a]	Median (95% CI)	n[b]	Median (95% CI)	
Age, years	378	54.8 (53.2–56.4)	154	55.7 (53.2–58.2)	0.55
BMI, kg/m^2	174	32.3 (31.2–33.4)	104	34.1 (32.3–35.2)	0.0954
HbA1c, %	174	6.2 (6.0–6.6)	103	6.3 (6.0–6.6)	0.8607
Insulin, mIU/L	172	20.0 (18.3–22.5)	103	26.0 (22.5–29.8)	0.0019
Homeostatic model assessment / insulin resistance	170	5.72 (4.85–6.88)	103	7.33 (6.61–8.35)	0.0023
ALT, U/L	378	42.0 (38.9–44.3)	153	69.4 (58.8–79.0)	<0.0001
AST, U/L	378	30.7 (29.7–32.4)	153	53.9 (49.2–56.1)	<0.0001
Alkaline phosphatase, U/L	378	74.8 (72.8–77.7)	153	77.8 (73.8–83.1)	0.0347
Bilirubin, mg/dL	377	0.38 (0.36–0.40)	153	0.44 (0.39–0.49)	0.0986
Platelet count, x10^3/mm^3	378	241 (233–247)	150	218 (208–233)	0.0008
High density lipoprotein, mg/dL	289	41.0 (40.0–44.0)	150	6.87 (6.50–7.30)	<0.0001

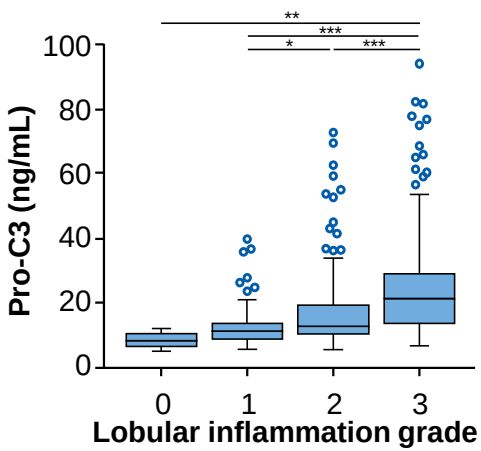
[a]378 samples were screened in this group; [b]154 samples were screened in this group

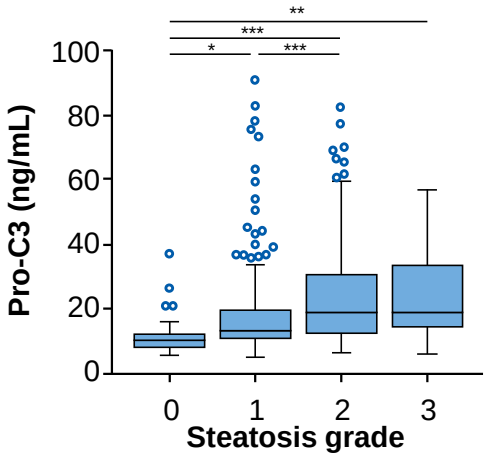
Pro-C3 is related to disease activity features and fibrosis











Box-and-whisker plots are shown as Tukey box plots
*p≤0.05; **p≤0.001; ***p<0.0001; †p<0.05 compared to all groups except 0, 1, 4, and 8; ††p<0.05 compared to 0, 5, and 7 and p<0.0001 compared to all other groups except 8; †††p<0.05 compared to 6 and p<0.0001 compared to all other groups except 8

Conclusions

- This exploratory analysis of the CENTAUR study demonstrated that **Pro-C3 can detect moderate and severe fibrosis in patients**, and can differentiate NASH from NAFL with fair accuracy
  - High baseline Pro-C3 correlates with markers of hepatocellular injury and insulin resistance
- **Pro-C3 has potential as a novel non-invasive screening tool** for selecting high-risk patients with NASH and fibrosis suitable for clinical trials
  - Replication in other cohorts could validate these findings
- There is ongoing investigation towards a multi-marker model, which includes Pro-C3, for improved sensitivity and specificity
  - Additional extracellular matrix neo-epitope markers and routine clinical markers may be included

Thank you

Back-up slides

Statistical analyses

- P values and multiple comparisons have been calculated using one-way ANOVA, with adjusted gender as a covariate
- Mann–Whitney U tests have been used to calculate differences between high and low Pro-C3 groups for numerical variables unless otherwise stated
- Chi-squared tests have been used to calculate differences between high and low Pro-C3 groups for categorical variables
- Correlations have been performed by Spearman’s rank correlation

A greater proportion of subjects with high Pro-C3 have higher grades / stages of ballooning, lobular inflammation, steatosis, and fibrosis

Category	Grade / stage	Pro-C3 <20.2 ng/mL (n=378), n (%)[a]	Pro-C3 >20.2 ng/mL (n=154), n (%)[b]	P value
Hepatocellular ballooning	0 1 2	154 (41) 145 (39) 79 (20)	17 (11) 43 (28) 94 (61)	<0.0001
Lobular inflammation	0 1 2 3	11 (3) 85 (22) 188 (50) 94 (25)	0 (0) 9 (6) 63 (41) 82 (53)	<0.0001
Steatosis	0 1 2 3	42 (11) 255 (67) 69 (18) 12 (3)	4 (3) 85 (55) 57 (37) 8 (5)	<0.0001
NAS	0 1 2 3 4 5 6 7 8	10 (2.5) 25 (7) 38 (10) 64 (17) 101 (27) 77 (20) 50 (13) 11 (3) 2 (0.5)	0 (0) 3 (2) 2 (1) 7 (5) 23 (15) 35 (23) 52 (34) 28 (18) 4 (2)	<0.0001
NASH CRN fibrosis	0 1 (1a+1b+1c) 2 3 4	112 (30) 119 (31) 76 (20) 62 (16) 9 (2)	8 (5) 26 (17) 36 (23) 65 (42) 19 (12)	<0.0001

[a]378 samples were screened in this group; [b]154 samples were screened in this group

Pro-C3 is related to disease activity features and fibrosis

Category	n[a]	Correlation coefficient (Pro-C3)	P value
Hepatocellular ballooning	532	0.458	<0.0001
Lobular inflammation	532	0.390	<0.0001
Steatosis	532	0.304	<0.0001
NAS	532	0.513	<0.0001
NASH CRN fibrosis	532	0.438	<0.0001

[a]532 samples were screened in this group

Demographics of patients by baseline Pro-C3

Category		Pro-C3 <20.2 ng/mL		Pro-C3 >20.2 ng/mL		P value
	Male, n (%)	201 (53)		70 (46)		0.1066
		n[a]	Median (95% CI)	n[b]	Median (95% CI)	
Serum biochemical levels	Albumin, g/dL	378	4.47 (4.43–4.51)	153	4.41 (0.35–4.47)	0.1061
	ALT, U/L	**378**	**42.0 (38.9–44.3)**	**153**	**69.4 (58.8–79.0)**	**<0.0001**
	AST, U/L	**378**	**30.7 (29.7–32.4)**	**153**	**53.9 (49.2–56.1)**	**<0.0001**
	Alkaline phosphatase, U/L	378	74.8 (72.8–77.7)	153	77.8 (73.8–83.1)	0.0347
	Bilirubin, mg/dL	377	0.38 (0.36–0.40)	153	0.44 (0.39–0.49)	0.0986
	Fasting plasma glucose, mg/dL	285	106 (104–109)	137	110 (106–115)	0.0967
	Platelet count, $x10^3/mm^3$	**378**	**241 (233–247)**	**150**	**218 (208–233)**	**0.0008**
	Mean hemoglobin, g/dL	378	14.5 (14.4–14.7)	150	14.6 (14.4–14.8)	0.5007
	HbA1c, %	174	6.2 (6.0–6.6)	103	6.3 (6.0–6.6)	0.8607
	Homeostatic model assessment / insulin resistance	**170**	**5.72 (4.85–6.88)**	**103**	**7.33 (6.61–8.35)**	**0.0023**
	Insulin, mIU/L	**172**	**20.0 (18.3–22.5)**	**103**	**26.0 (22.5–29.8)**	**0.0019**
	White blood cells, $x10^3/mm^3$	378	6.80 (6.60–7.00)	150	6.87 (6.50–7.30)	0.7398
Lipids	Cholesterol, mg/dL	378	188 (185–195)	153	191 (181–200)	0.9658
	Low density lipoprotein, mg/dL	289	117 (113–122)	129	122 (111–128)	0.8784
	Very low density lipoprotein, mg/dL	299	29.0 (27.0–31.0)	134	31.0 (27.0–33.0)	0.1808
	High density lipoprotein, mg/dL	**289**	**41.0 (40.0–44.0)**	**150**	**6.87 (6.50–7.30)**	**<0.0001**
	Triglycerides, mg/dL	377	148 (140–157)	153	153 (139–169)	0.1380
Metabolic factors	Documented type 2 diabetes, n (%)	149 (39)		67 (44)		0.3843
	BMI, kg/m^2	174	32.3 (31.2–33.4)	104	34.1 (32.3–35.2)	0.0954
	Height, inches	174	168 (165–169)	104	169 (166–171)	0.4469
	Waist circumference, inches	171	42.1 (41.2–43.3)	104	44.1 (41.7–45.3)	0.0693
	Weight, kg	175	92.0 (89.0–93.9)	106	98.1 (82.0–115)	0.1008

[a]378 samples were screened in this group; [b]154 samples were screened in this group