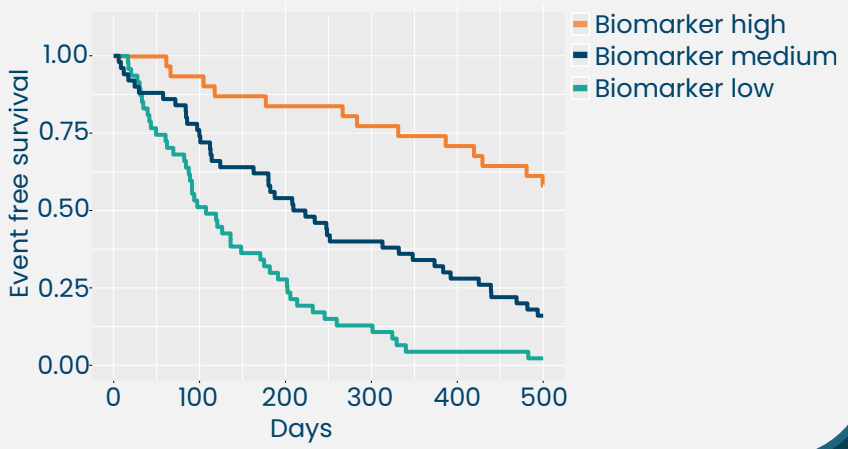
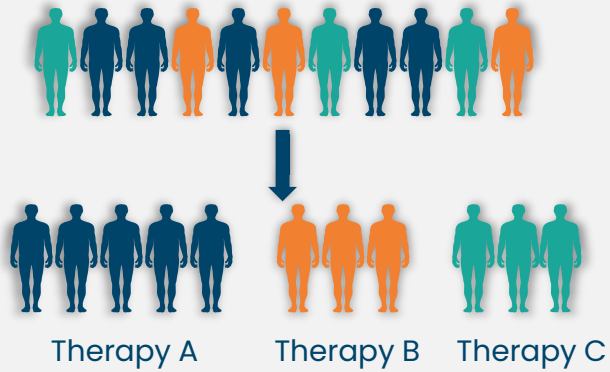
Biomarkers is biological crystal bowl

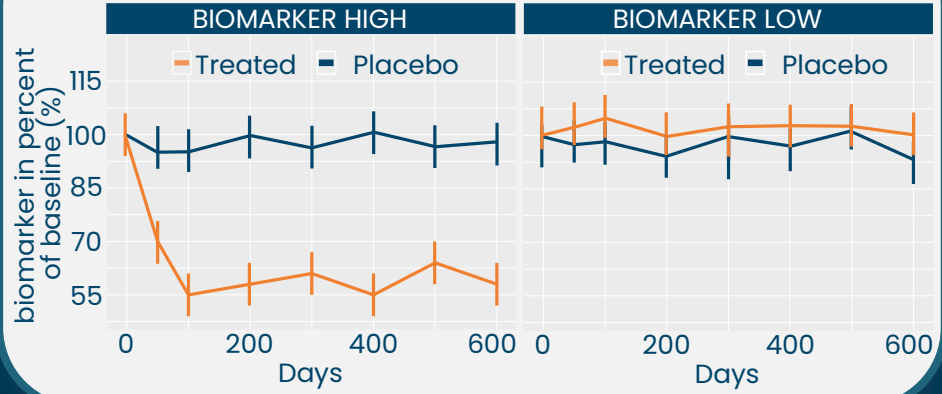
Disease severity and progression



Diagnosis of subtypes



Response and efficacy



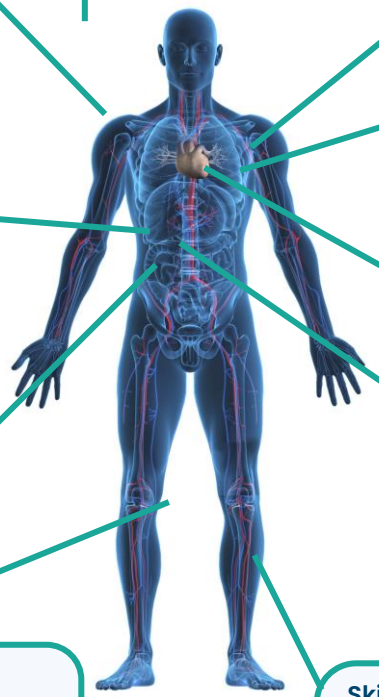
NORDIC BIOSCIENCE

Demolition & Repair 24-7

You are not the same you were Monday morning

Disrupted collagen/tissue balance

Fibrosis and extracellular matrix remodeling are essential for many pathologies



Brain
1. Alzheimers
2. Traumatic brain injury
3. Parkinsons disease
4. Dimentias

Systemic
1. Systemic sclerosis

Muscle
1. Dermatomyositis
2. Polymyositis
3. Myositis
4. Cachexia
5. Sarcopenia

Lung
1. Asthma
2. COPD
3. PAH
4. Interstitial lung disease

Liver
1. NASH
2. Alcoholic liver disease
3. Portal hypertension
4. Hepatitis B/C
5. Primary sclerosing cholangitis
6. Primary biliary cirrhosis

Cardiovasulcar
1. Atherosclerosis
2. Myocardial infarction
3. Cardiac fibrosis
4. Atrial fibrillation

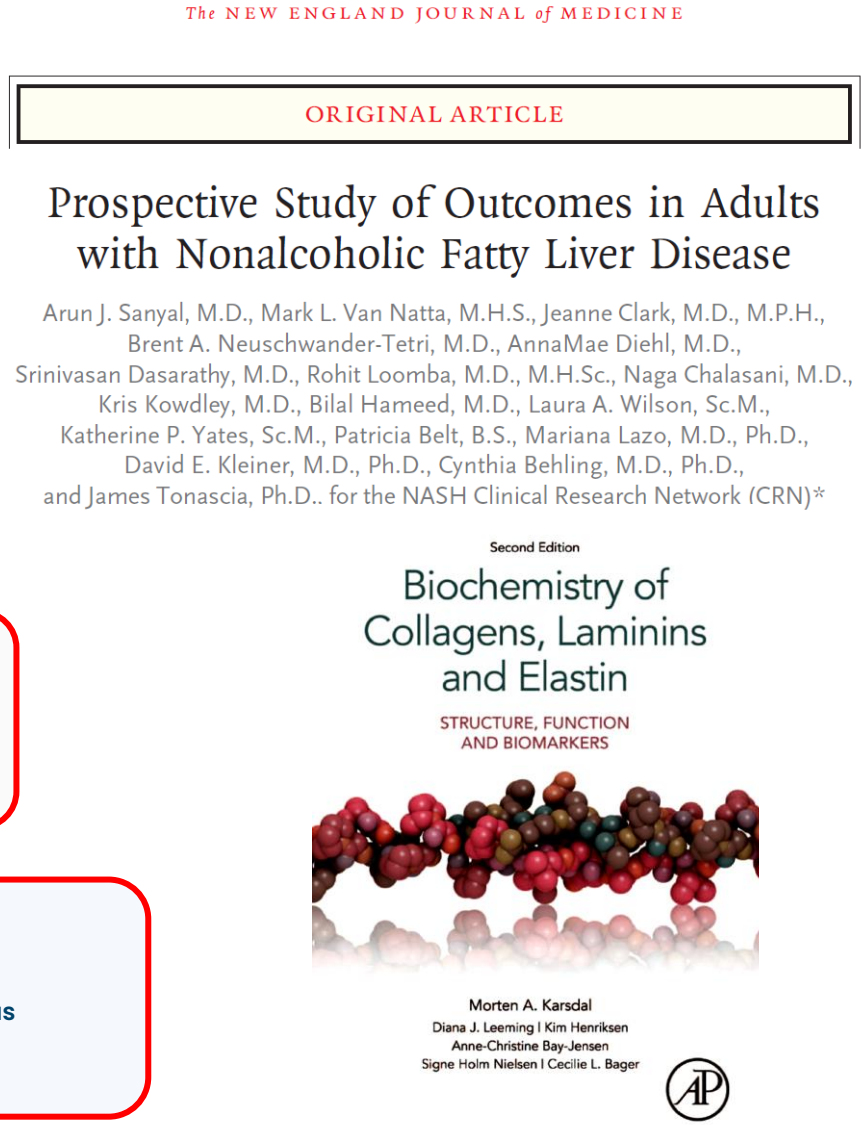
Intestine
1. Crohn's disease
2. Ulcerative colitis
3. Intestinal fibrosis
4. Intestinal fistula
5. Irritable bowel disease
6. Hernia

Kidney
1. Diabetic nephropathy
2. Glomerular sclerosis
3. Chronic allograf nephropathy
4. IgA nephropathy
5. Lupus nephritis

Bone and cartilage
1. Osteoporosis
2. Osteoarthritis
3. Rheumatoid arthritis
4. Spondyloarthritis
5. Psoriatic arthritis

Skin
1. Atopic dermatitis
2. Hypertrophic scars
3. Keloids
4. Psoriasis
5. Scleroderma
6. Lupus

Cancers
1. HCC
2. Pancreas



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Prospective Study of Outcomes in Adults with Nonalcoholic Fatty Liver Disease

Arun J. Sanyal, M.D., Mark L. Van Natta, M.H.S., Jeanne Clark, M.D., M.P.H., Brent A. Neuschwander-Tetri, M.D., AnnaMae Diehl, M.D., Srinivasan Dasarathy, M.D., Rohit Loomba, M.D., M.H.Sc., Naga Chalasani, M.D., Kris Kowdley, M.D., Bilal Hameed, M.D., Laura A. Wilson, Sc.M., Katherine P. Yates, Sc.M., Patricia Belt, B.S., Mariana Lazo, M.D., Ph.D., David E. Kleiner, M.D., Ph.D., Cynthia Behling, M.D., Ph.D., and James Tonascia, Ph.D.. for the NASH Clinical Research Network (CRN)*

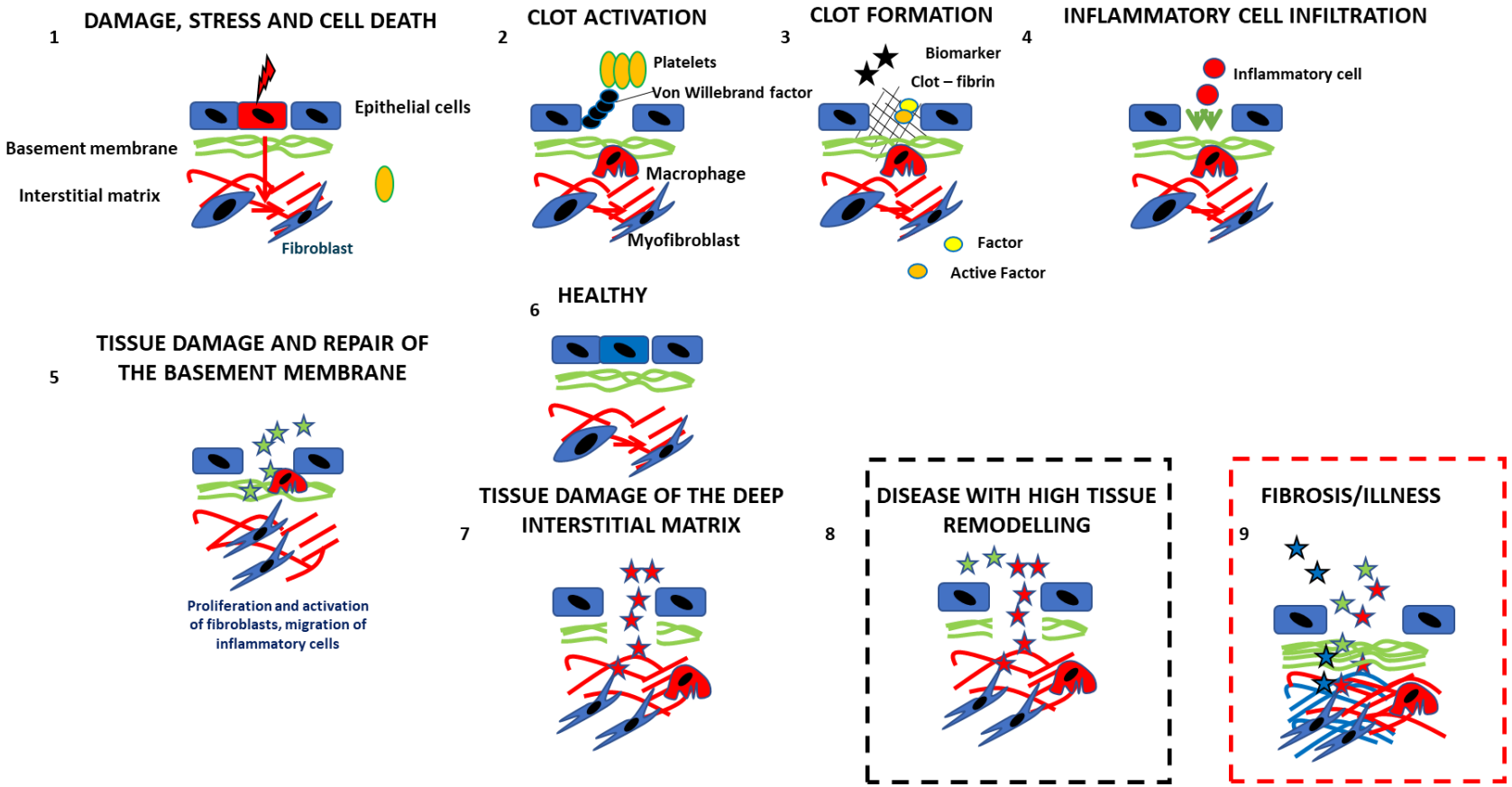
Second Edition

Biochemistry of Collagens, Laminins and Elastin

STRUCTURE, FUNCTION AND BIOMARKERS

Morten A. Karsdal
Diana J. Leeming | Kim Henriksen
Anne-Christine Bay-Jensen
Signe Holm Nielsen | Cecilie L. Bager

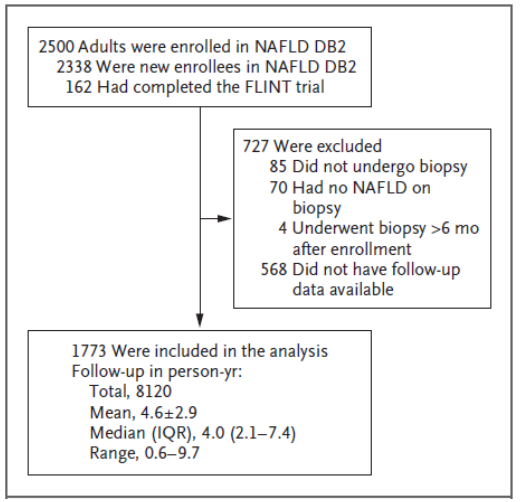
What is elevated tissue remodelling? the common denominator among chronic diseases



Karsdal MA et al, Autoimmunity reviews, 2021

NASH: Not only a liver disease! An organ death race!

Events/100	Total population	F0-F2	F3	F4
Hypertension	7.76	6.5	12.17	14.49
Diabetes	4.84	4.45	6.24	7.53
Kidney	2.53	2.17	2.97	4.49
CVD- events	0.83	0.8	0.93	0.81
Non-liver cancer	0.82	0.73	1.03	1.00
Death Any Cause	0.57	0.32	0.89	1.76
Liver-related events	0.46	0.05	0.99	2.69
Liver-related deaths	0.15	0.04	0.28	0.68
HCC	0.11	0.04	0.34	0.14

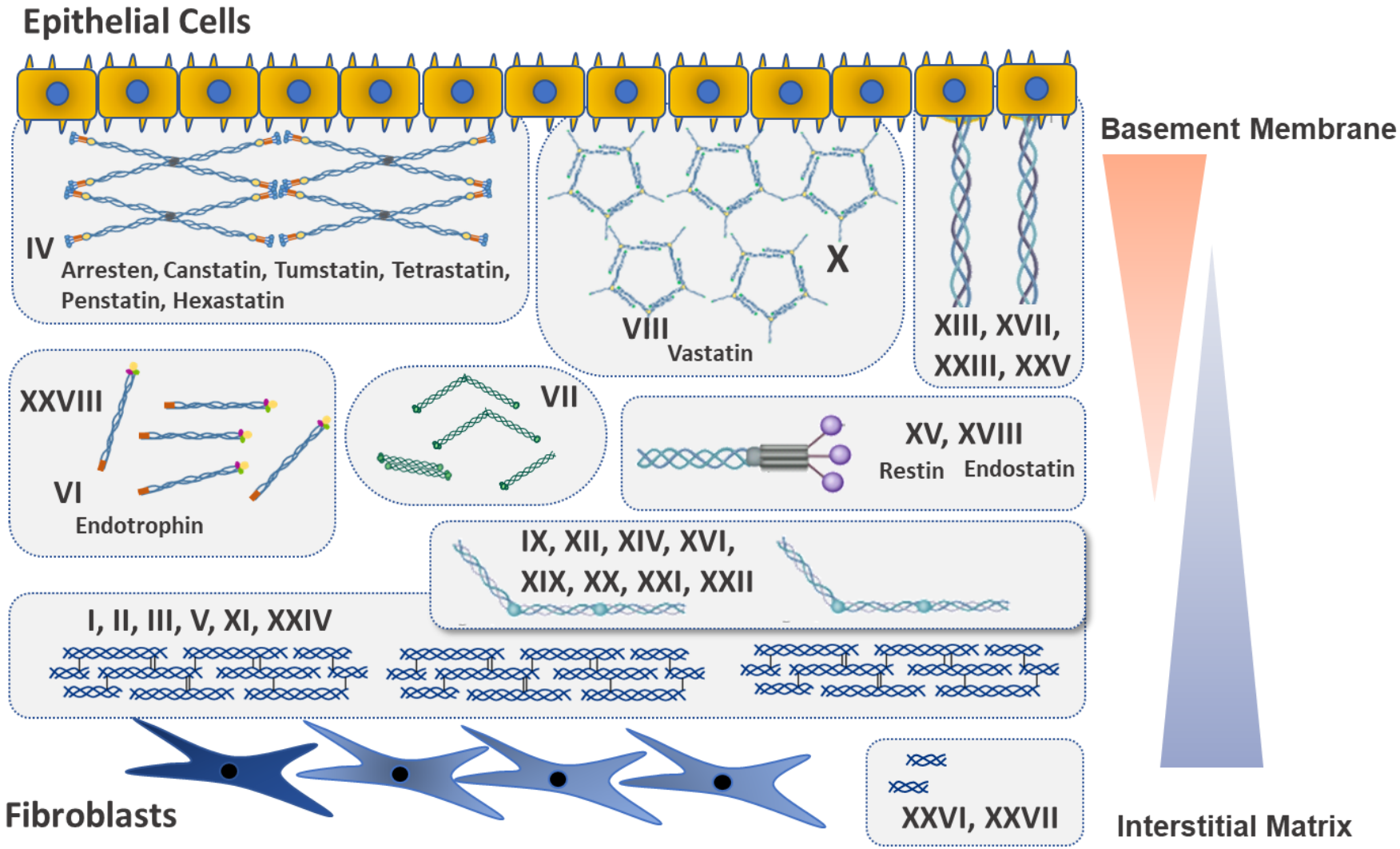


Sanyal A. et al, NEJM, 385, 2021

n=1773. 4.6 years follow up

NORDIC BIOSCIENCE

Collagen Biomarkers

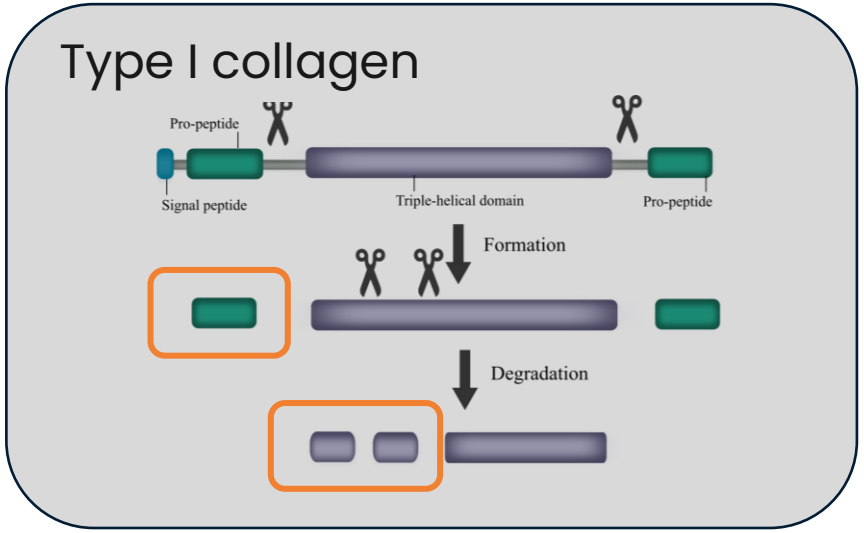


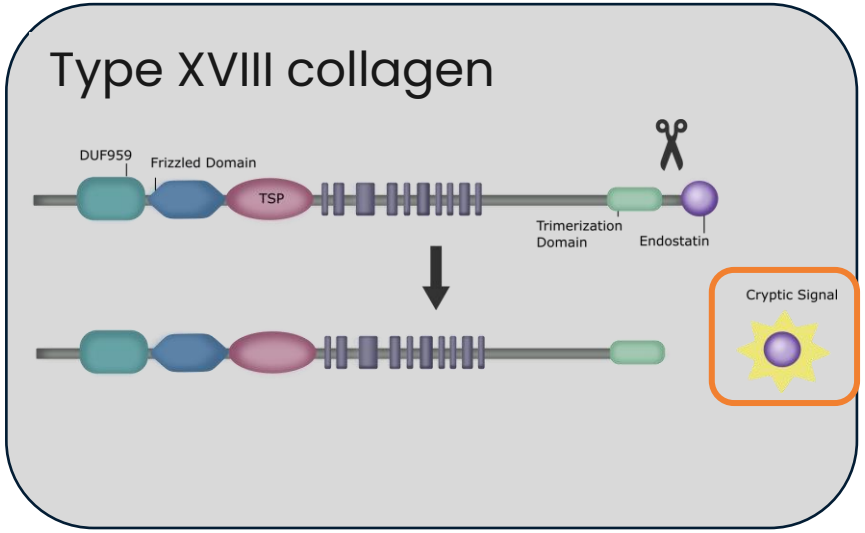
NORDIC BIOSCIENCE

Proteins have many different domains – selection of the right epitope

Each domain may have unique functions:

- Some are released during tissue formation
- Some are released during tissue degradation
- Some are cryptic signals being released during tissue turnover



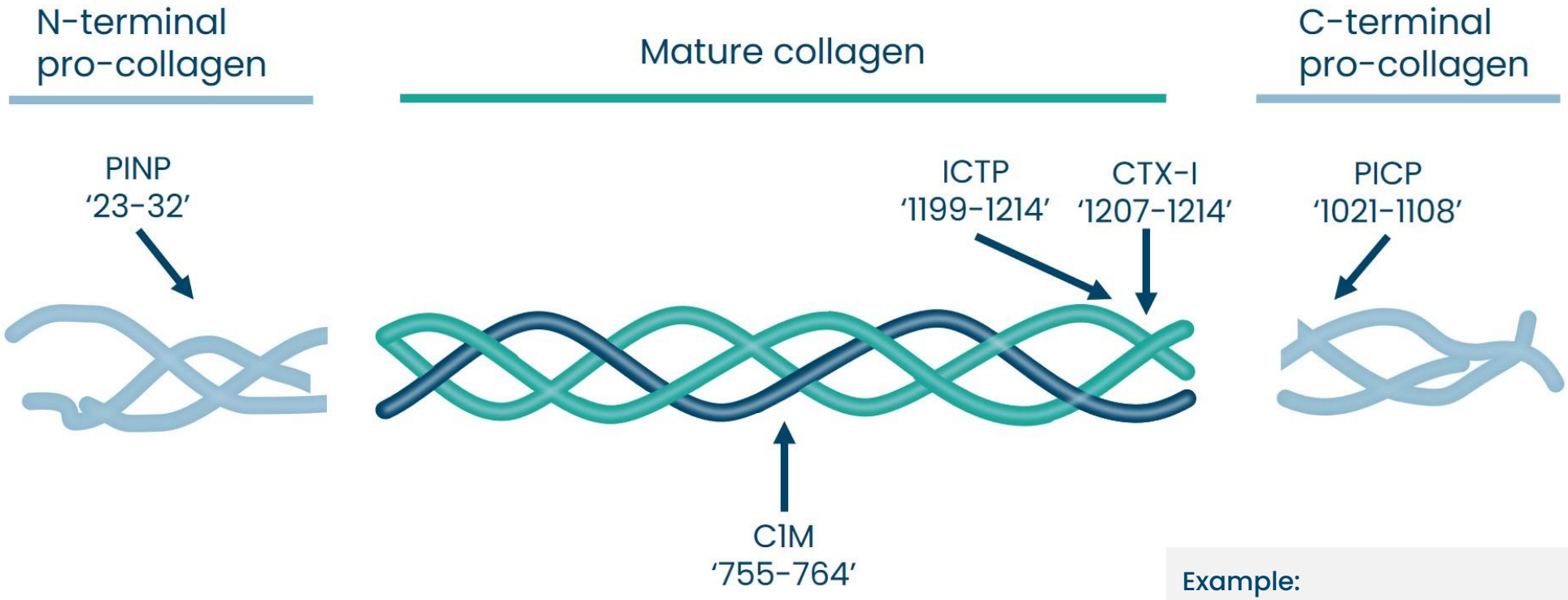


Karsdal MA et al, The collagen book

NORDIC BIOSCIENCE

We should not just measure proteins – but epitopes

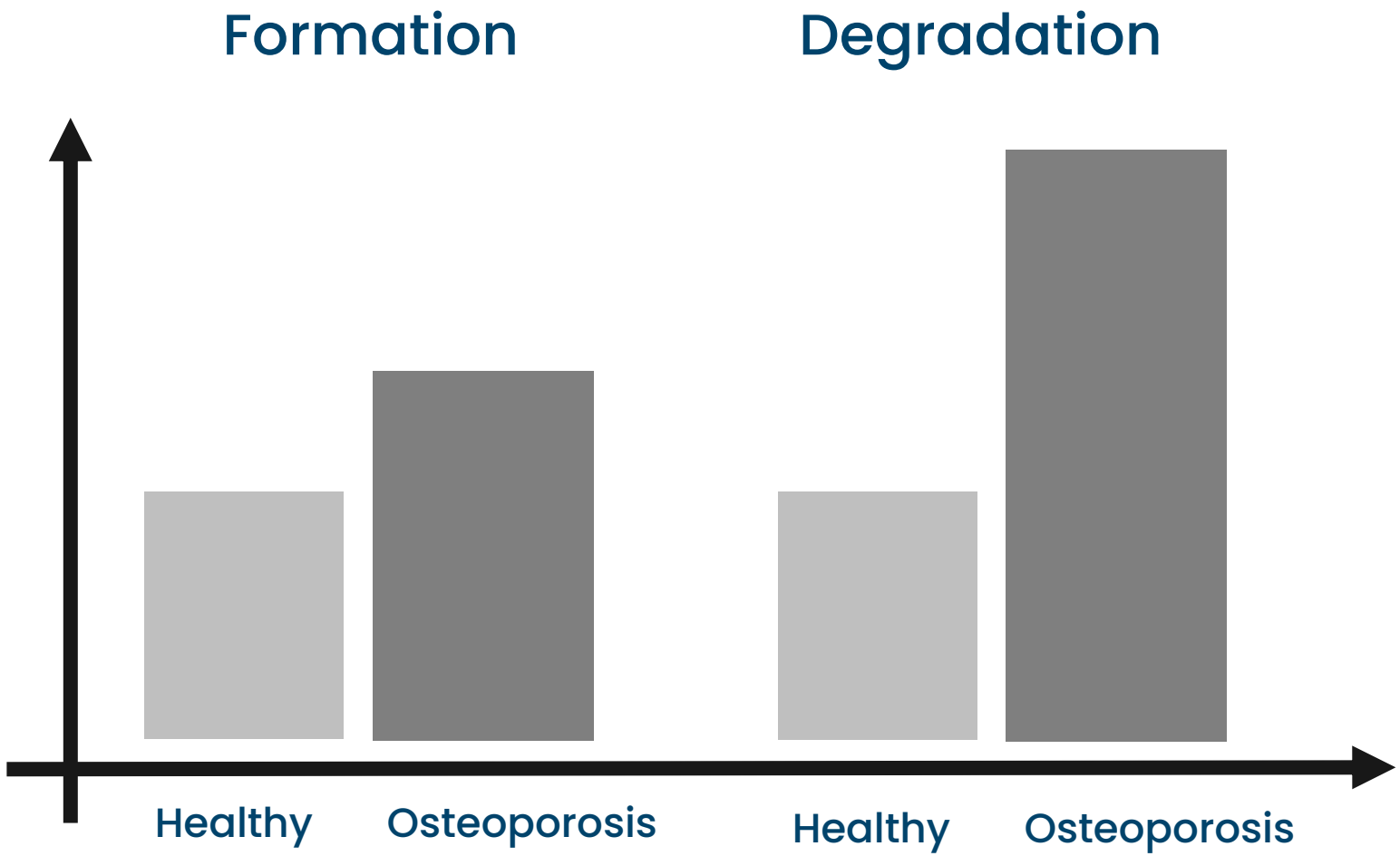
There are many different cleavage fragments of type I collagen – each providing unique information on tissue turnover



Example:
CTX-I = bone resorption
C1M = soft-tissue degradation

Nordic Bioscience

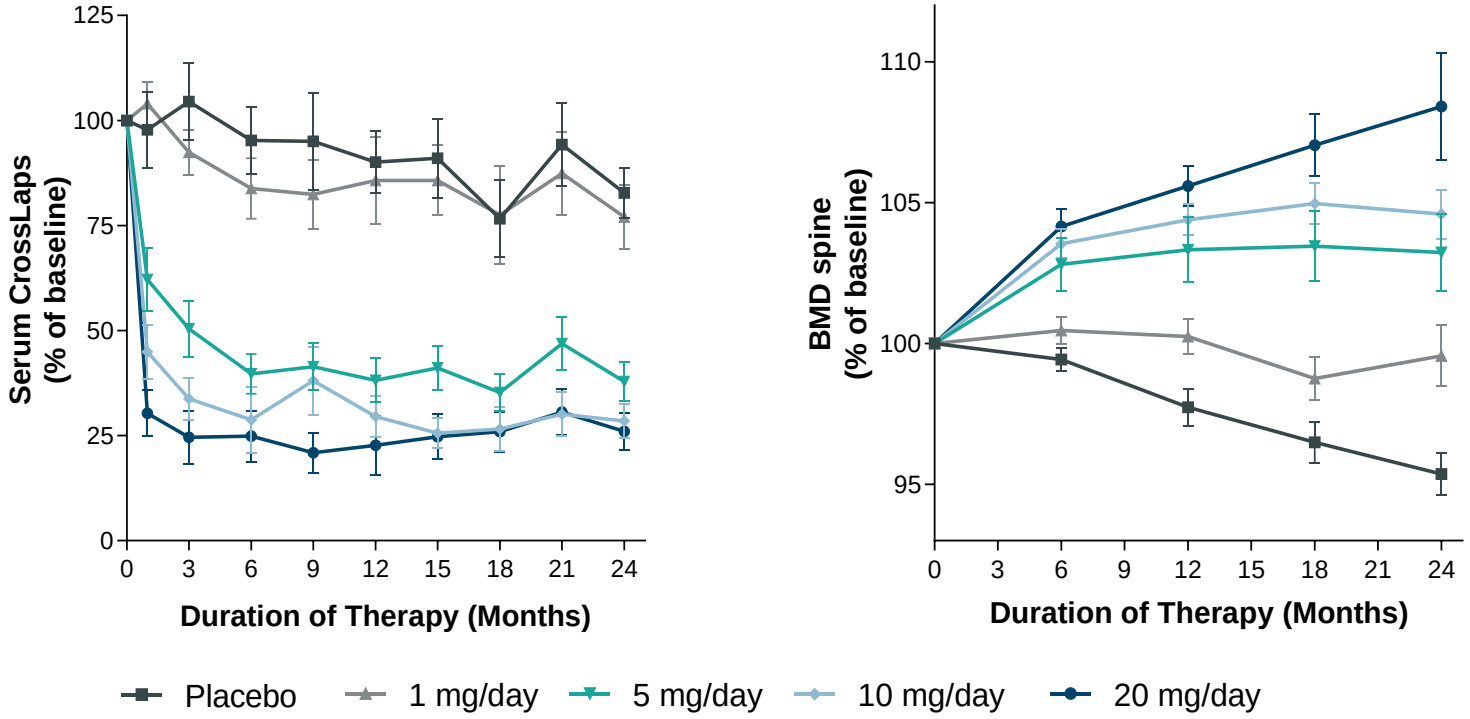
The tissue balance was changed



NORDIC BIOSCIENCE

Precision Medicine – "The Beginning" – early efficacy

Liver, Lung, Skin (SSC), Heart, IBD, Kidney and Cancer



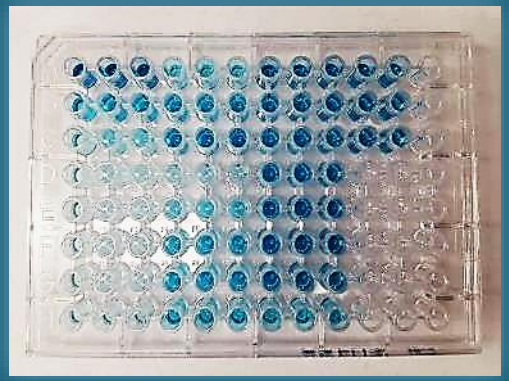
NORDIC BIOSCIENCE

Filling the biomarker gap for the future

Discovery

10.000 Ideas

Refinement



200 Assays

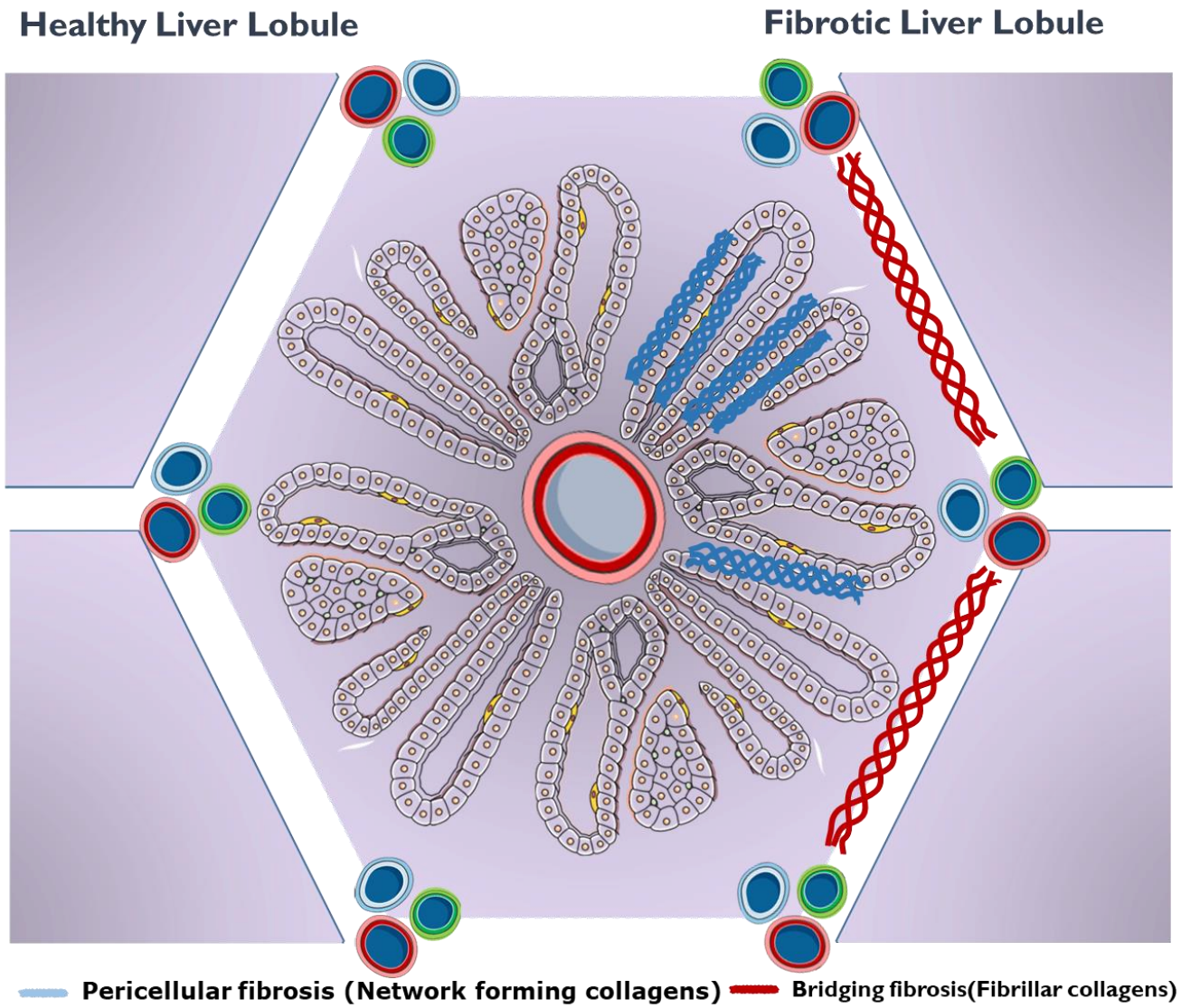
Robustness

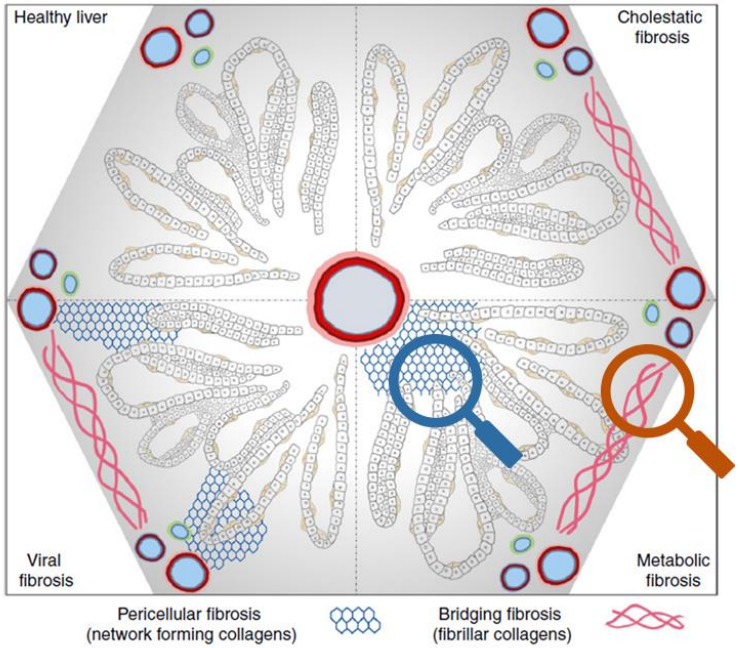
50 Assays

World Wide

15 Assays

Fibrosis is not just fibrosis!





Basement membrane formation → Reparative response

Interstitial matrix → Dense matrix indicative of late stage disease

Karsdal et al. , Hepatology 2020

Research Article
NAFLD and Alcohol-Related Liver Diseases

JOURNAL OF HEPATOLOGY

Complexity of ballooned hepatocyte feature recognition: Defining a training atlas for artificial intelligence-based imaging in NAFLD

Elizabeth M. Brunt[1,*,†], Andrew D. Clouston[2], Zachary Goodman[3], Cynthia Guy[4], David E. Kleiner[5], Carolin Lackner[6], Dina G. Tiniakos[7,8], Aileen Wee[9], Matthew Yeh[10], Wei Qiang Leow[11], Elaine Chng[12], Yayun Ren[12], George Goh Boon Bee[13], Elizabeth E. Powell[14,15], Mary Rinella[16], Arun J. Sanyal[17], Brent Neuschwander-Tetri[18], Zobair Younossi[19], Michael Charlton[20], Vlad Ratziu[21], Stephen A. Harrison[22,23], Dean Tai[11,*,†], Quentin M. Anstee[7,24,*,†]

Background & Aims: Histologically assessed hepatocyte ballooning is a key feature discriminating non-alcoholic steatohepatitis (NASH) from steatosis (NAFL). Reliable identification underpins patient inclusion in clinical trials and serves as a key regulatory-approved surrogate endpoint for drug efficacy. High inter/intra-observer variation in ballooning measured using the NASH CRN semi-quantitative score has been reported yet no actionable solutions have been proposed.

Methods: A focused evaluation of hepatocyte ballooning recognition was conducted. Digitized slides were evaluated by 9 internationally recognized expert liver pathologists on 2 separate occasions: each pathologist independently marked every ballooned hepatocyte and later provided an overall non-NASH NAFL/NASH assessment. Interobserver variation was assessed and a 'concordance atlas' of ballooned hepatocytes generated to train second harmonic generation/two-photon excitation fluorescence imaging-based artificial intelligence (AI).

Results: The Fleiss kappa statistic for overall interobserver agreement for presence/absence of ballooning was 0.197 (95% CI 0.094–0.300), rising to 0.362 (0.258–0.465) with a ≥5-cell threshold. However, the intraclass correlation coefficient for consistency was higher (0.718 [0.511–0.900]), indicating 'moderate' agreement on ballooning burden. 133 ballooned cells were identified using a ≥5/9 majority to train AI ballooning detection (AI-pathologist pairwise concordance 19–42%, comparable to inter-pathologist pairwise concordance of between 8–75%). AI quantified change in ballooned cell burden in response to therapy in a separate slide set.

Conclusions: The substantial divergence in hepatocyte ballooning identified amongst expert hepatopathologists suggests that ballooning is a spectrum, too subjective for its presence or complete absence to be unequivocally determined as a trial endpoint. A concordance atlas may be used to train AI assistive technologies to reproducibly quantify ballooned hepatocytes that standardize assessment of therapeutic efficacy. This atlas serves as a reference standard for ongoing work to refine how ballooning is classified by both pathologists and AI.

Lay summary: For the first time, we show that, even amongst expert hepatopathologists, there is poor agreement regarding

Keywords: Nonalcoholic fatty liver disease; nonalcoholic steatohepatitis; NASH; NAFLD; Ballooning; Artificial intelligence; Machine learning; Histology.
Received 22 September 2021; received in revised form 21 December 2021; accepted 7 January 2022; available online 25 January 2022
* Corresponding authors. Addresses: Translational & Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Fourth Floor, William Leech Building, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK; Tel.: + 44 (0) 191 208 7012. (Q.M. Anstee), or Campus Box 8118, 660 S Euclid Avenue, Washington University School of Medicine, St Louis, MO 63110, USA; Tel.: +1- 314-273-7805. (E.M. Brunt: Emeritus Professor), or Histoindex Ltd., 79 Ayer Rajah Crescent, #04-05, JTC Launchpad 139955, Singapore; Tel.: +65 6774 4990. (D. Tai).
E-mail addresses: quentin.anstee@newcastle.ac.uk (Q.M. Anstee), ebrunt@wustl.edu (E.M. Brunt), dean.tai@histoindex.com (D. Tai).
† Joint senior and corresponding authors.
https://doi.org/10.1016/j.jhep.2022.01.011

JOURNAL OF HEPATOLOGY
The Home of Liver Research
EASL

RESEARCH ARTICLE | VOLUME 73, ISSUE 6, P1322-1332, DECEMBER 01, 2020

Suboptimal reliability of liver biopsy evaluation has implications for randomized clinical trials

Beth A. Davison • Stephen A. Harrison • Gad Cotter • ... Julie Iwashita • Gary G. Koch • Howard C. Dittrich • Show all authors

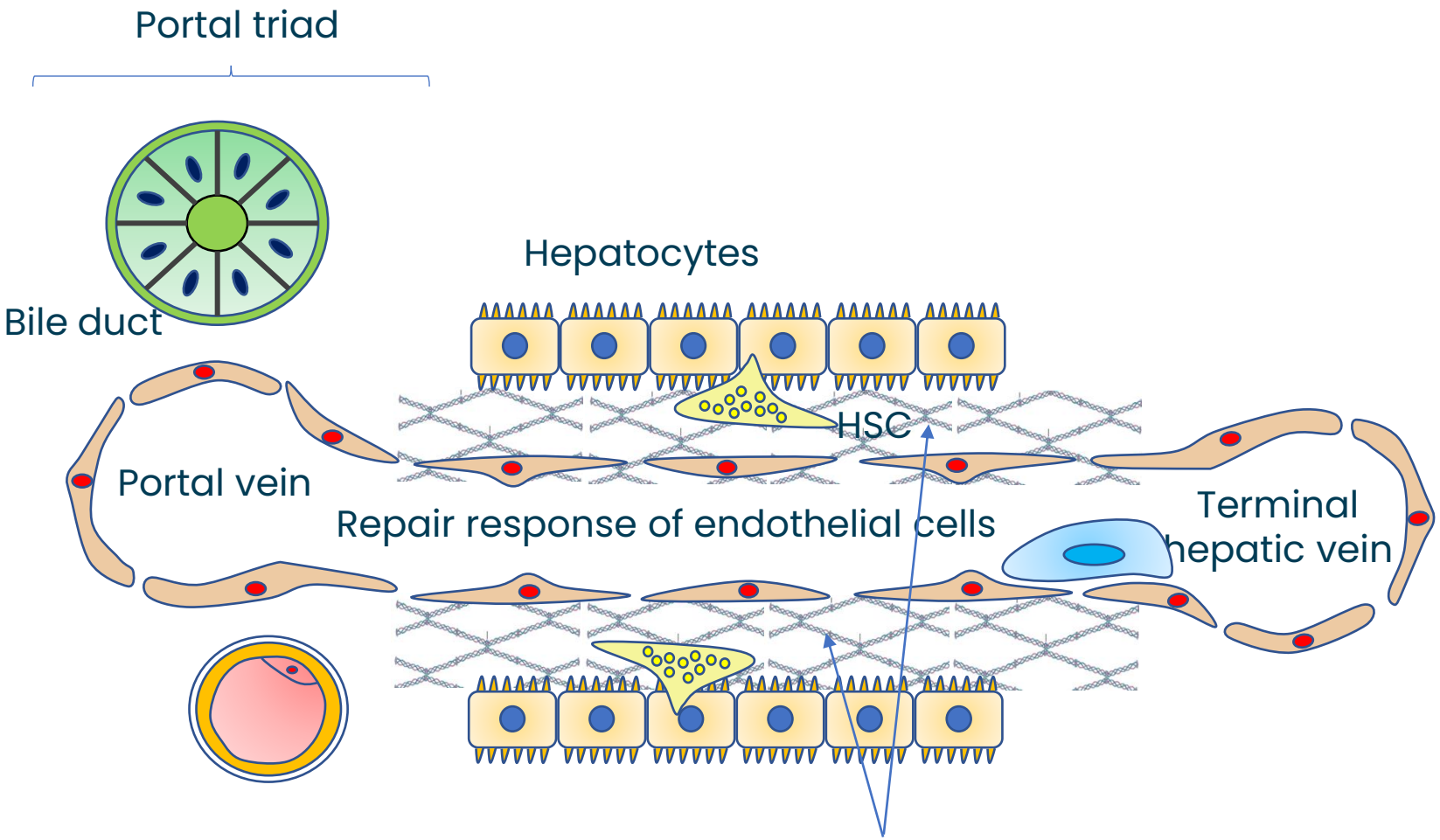
Published: June 27, 2020 • DOI: https://doi.org/10.1016/j.jhep.2020.06.025 • Check for updates

Results

Inter-reader linearly weighted kappas were 0.609, 0.484, 0.328, and 0.517 for steatosis, fibrosis, lobular inflammation, and ballooning, respectively. Inter-reader unweighted kappas were 0.400 for the diagnosis of NASH, 0.396 for NASH resolution without worsening fibrosis, and 0.366 for fibrosis improvement without worsening NASH. In the current study, 46.3% of the patients included in the study based on 1 hepatopathologist's qualifying reading were deemed not to meet the study's histologic inclusion criteria by at least 1 of the 3 hepatopathologists. The MSDC-0602K treatment effect was lowest for those histologic features with lower inter-reader reliability. Simulations show that the lack of reliability of endpoints and inclusion criteria can drastically reduce study power – from >90% in a well-powered study to as low as 40%.

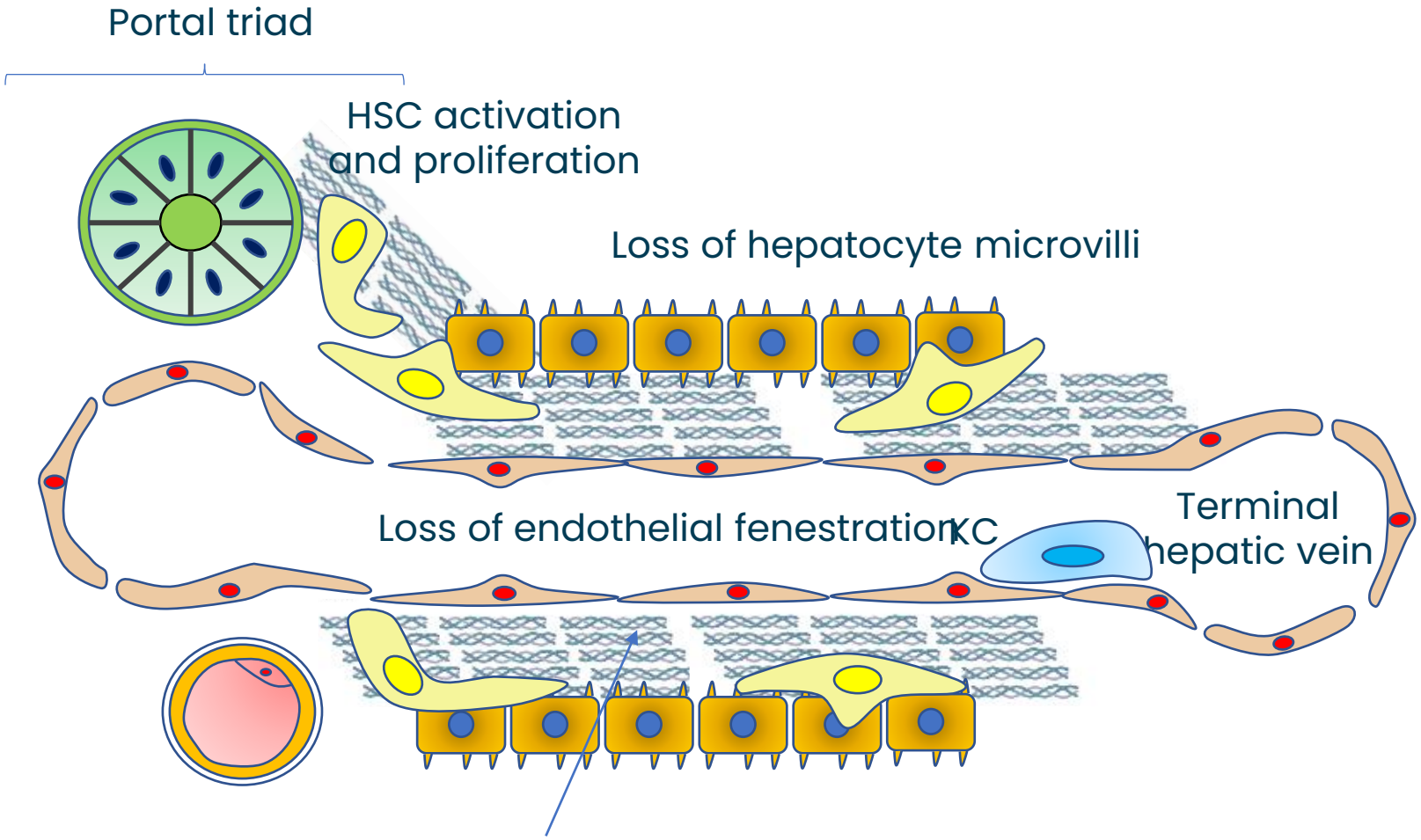
Conclusions

Early Regenerative Phase



Karsdal et al., Hepatology, 2019

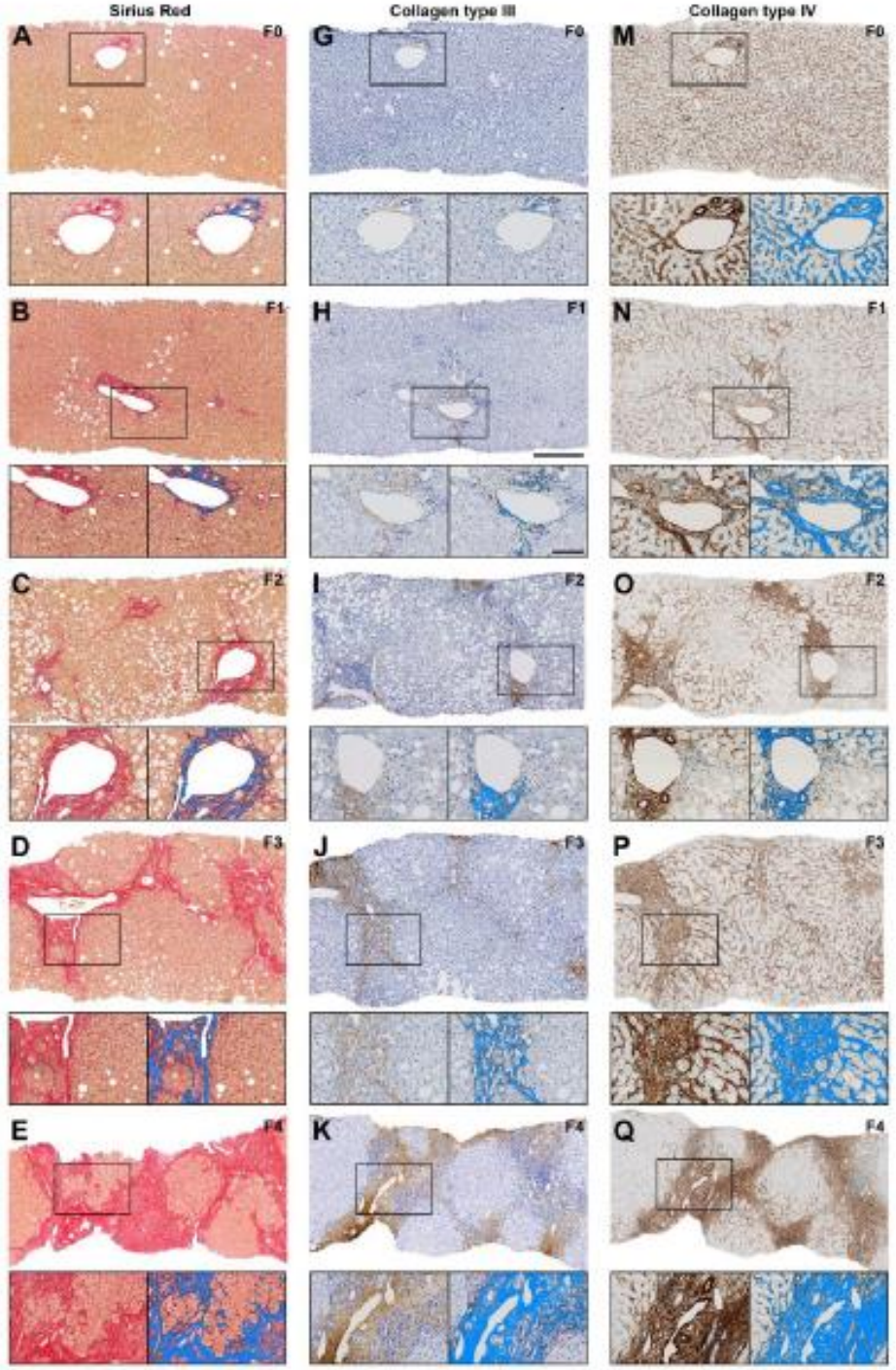
Advanced Liver Fibrosis

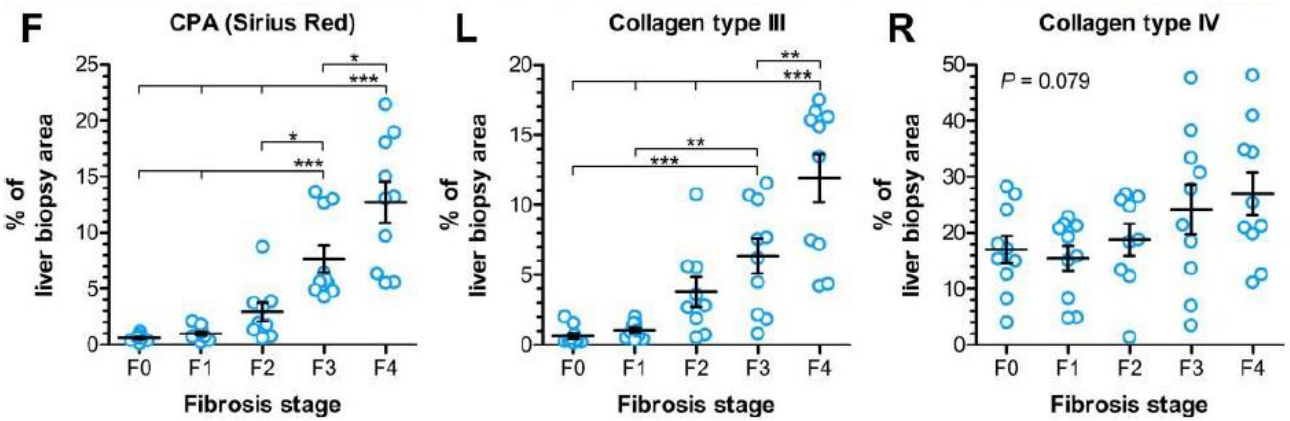


Karsdal et al., Hepatology, 2019

Nordic Bioscience

Which collagens are in the liver ?





Pathology - Research and Practice 231 (2022) 153798

Contents lists available at ScienceDirect

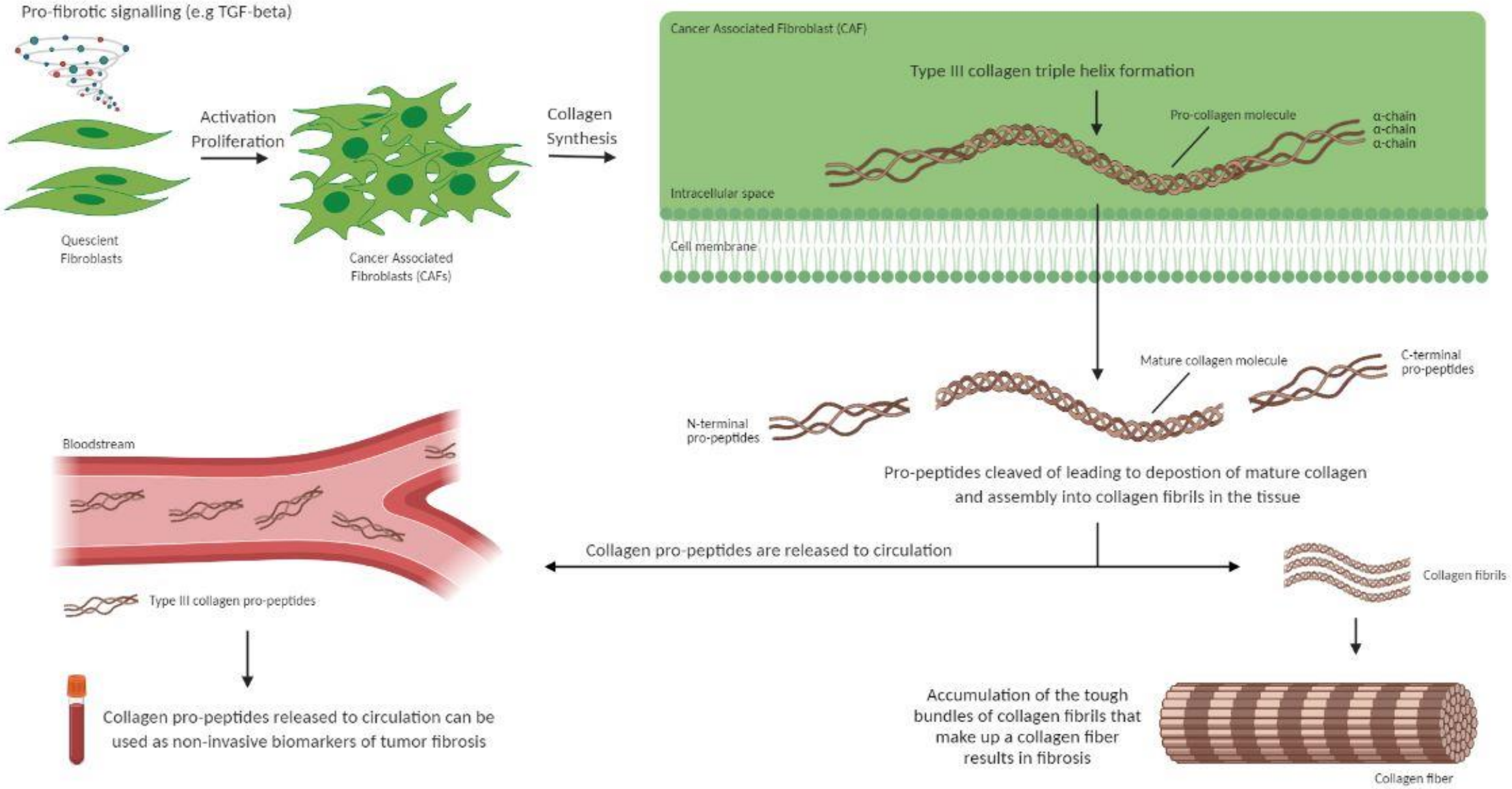
Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

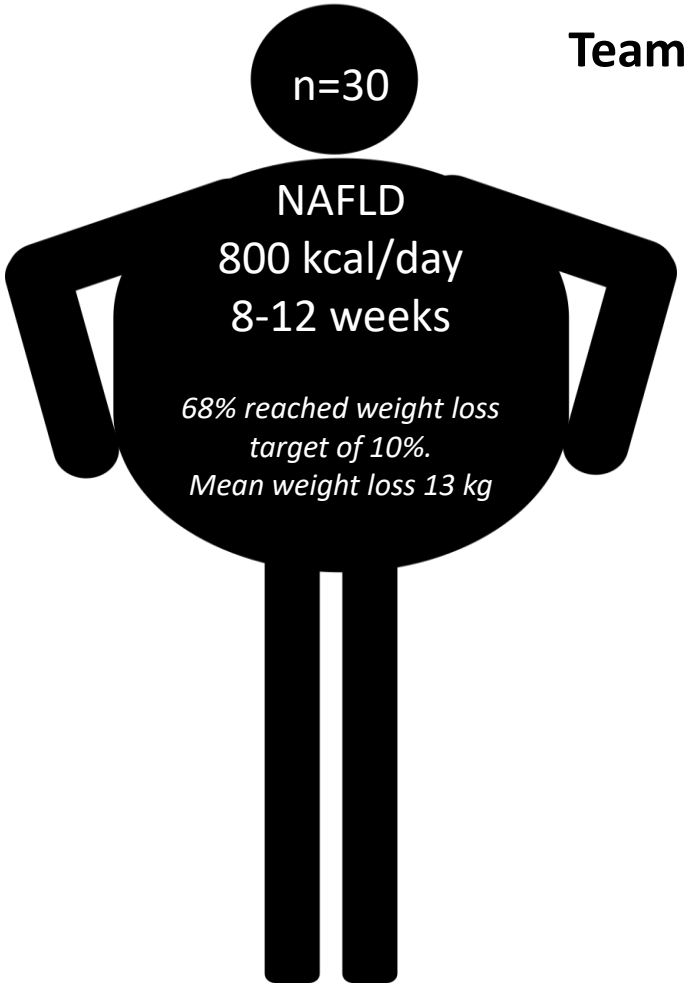
Stage-dependent expression of fibrogenic markers in alcohol-related liver disease

Mia Dahl Sørensen [a,b], Maja Thiele [b,c], Aleksander Krag [b,c], Samuel Joseph Daniels [d], Diana Julie Leeming [d], Morten Karsdal [d,e], Sönke Detlefsen [a,b,*]

[a] Department of Pathology, Odense University Hospital, Odense, Denmark
[b] Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
[c] Fibrosis, Fatty Liver and Steatohepatitis Research Centre Odense (FLASH), Department of Gastroenterology and Hepatology, Odense University Hospital, Odense, Denmark
[d] Nordic Bioscience A/S, Herlev Hovedgade, Herlev, Denmark
[e] Department of Molecular Medicine, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark


Pro-fibrotic signalling (e.g TGF-beta)
Activation
Proliferation
Quescient
Fibroblasts
Cancer Associated
Fibroblasts (CAFs)
Collagen
Synthesis
Cancer Associated Fibroblast (CAF)
Type III collagen triple helix formation
Pro-collagen molecule
α-chain
α-chain
α-chain
Intracellular space
Cell membrane
Mature collagen molecule
C-terminal
pro-peptides
N-terminal
pro-peptides
Pro-peptides cleaved of leading to depostion of mature collagen
and assembly into collagen fibrils in the tissue
Collagen pro-peptides are released to circulation
Collagen fibrils
Bloodstream
Type III collagen pro-peptides
Collagen pro-peptides released to circulation can be
used as non-invasive biomarkers of tumor fibrosis
Accumulation of the tough
bundles of collagen fibrils that
make up a collagen fiber
results in fibrosis
Collagen fiber

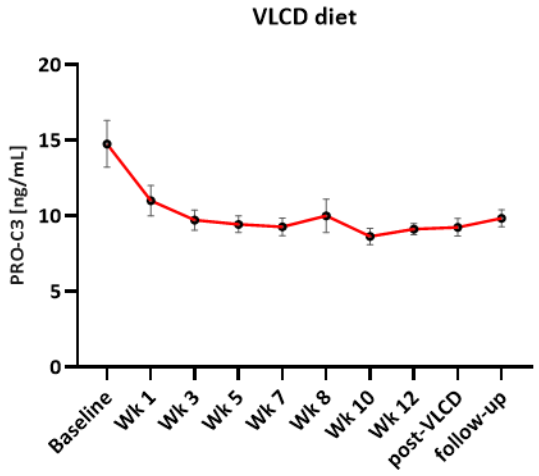
DYNAMICS OF BIOMARKERS

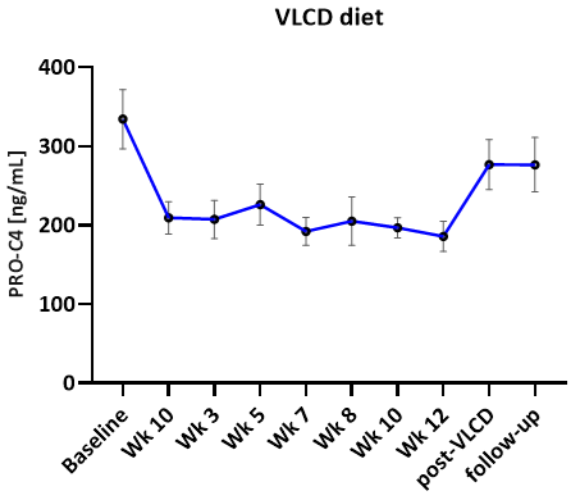


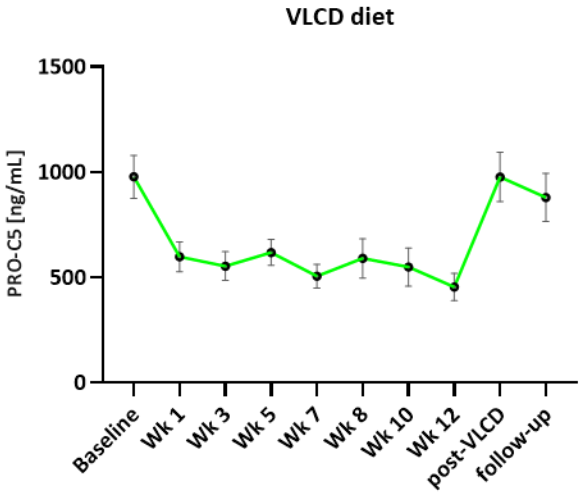
Team Quentin

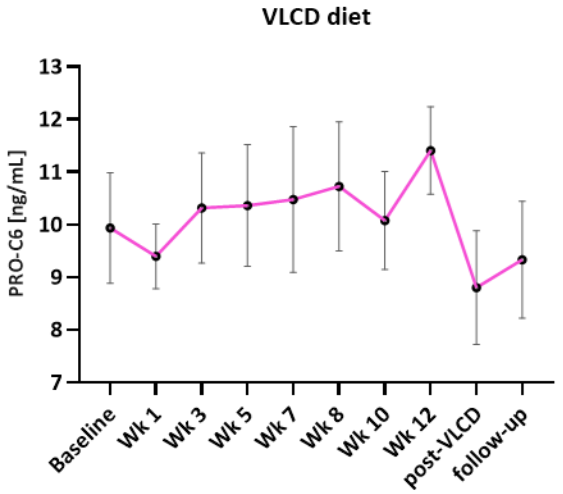
Subject Characteristics	Baseline (n=30)	Post-VLCD (n=27)	P-Value
Age (years)	56 ± 12		
Sex (n) male/female	18/12		
Time since NAFLD Diagnosis (months): Mean Median (range)	28.4 ± 31.7 13.5 (1-113)		
Anthropometry			
Weight (kg)	119 ± 25	104 ± 21	0.000**
BMI (kg/m²)	42 ± 8	37 ± 8	0.000**
Body fat (%)	45 ± 6.9	40 ± 9.1	0.001**
Blood pressure: Systolic (mmHg)	144 ± 15	133 ± 14	0.003**
Diastolic (mmHg)	86 ± 11	81 ± 9	0.018*
Blood samples			
Total cholesterol (mmol/L)	4.3 ± 0.9	4.3 ± 1.1	0.652
Triglycerides (mmol/L)	2.1 ± 1.8	2.0 ± 1.4	0.156
HDL (mmol/L)	1.2 ± 0.3	1.6 ± 1.9	0.270
AST (IU/L)	35 ± 18	25 ± 9	0.004**
ALT (IU/L)	47 ± 30	31 ± 16	0.003**
GGT (IU/L)	82 ± 74	52 ± 72	0.000**
Fasting glucose (mmol/L)	7.5 ± 2.3	6.1 ± 1.1	0.002**
Hba1c (mmol/mol)	50 ± 13	42 ± 9	0.000**
Insulin (pmol/L)	135 ± 85	92 ± 91	0.018*
Fibroscan			
Stiffness (KPa)	13.0 ± 6.6	8.0 ± 2.9	0.022*
IQR (KPa)	3.5 ± 3.0	2.5 ± 2.8	0.183
Non-invasive scores			
FIB-4	1.5 ± 1.0	1.2 ± 0.7	0.206
QRISK2	15.6 ± 14.2	11.9 ± 9.8	0.030*

Dynamics of biomarkers



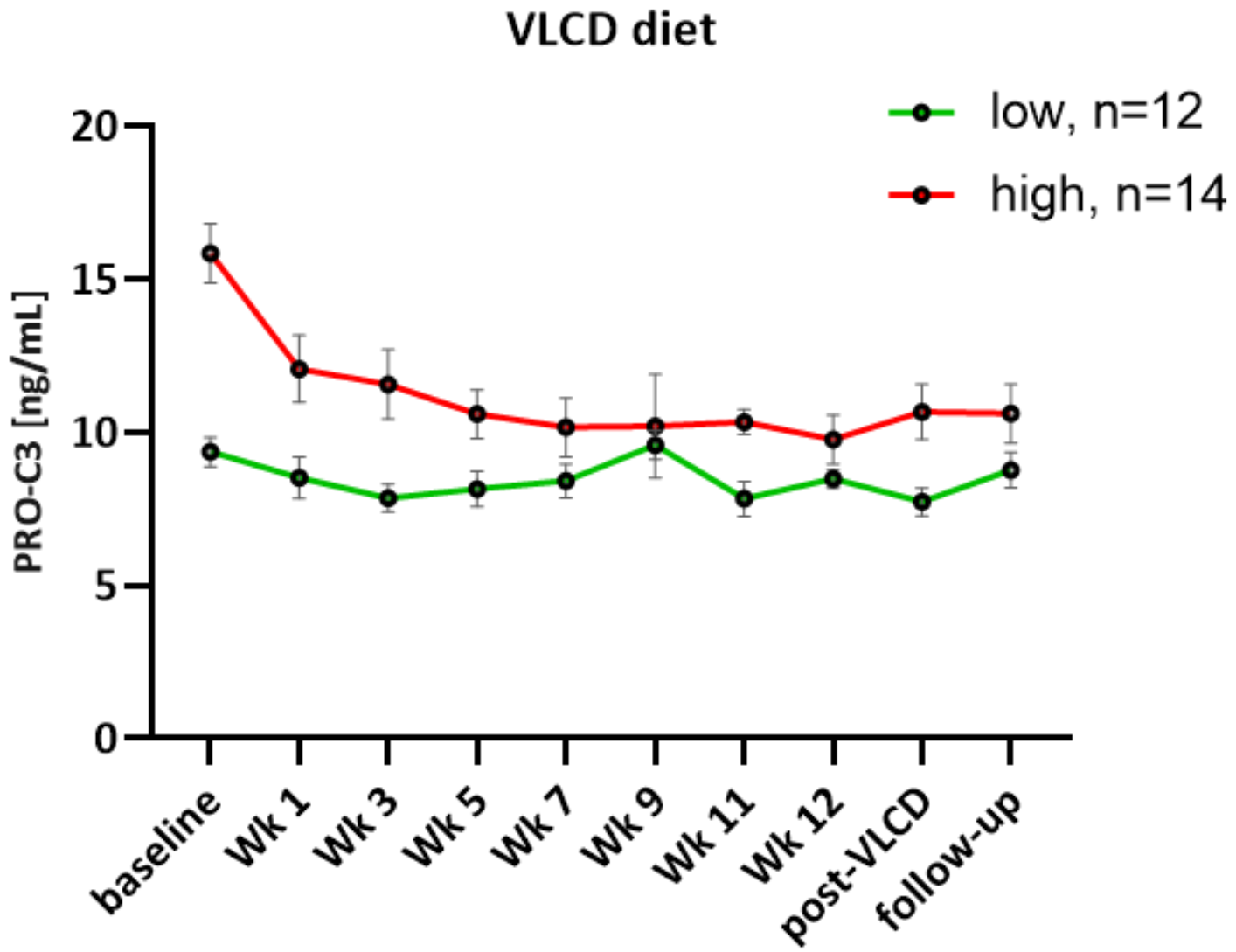






Mean with SEM

A low formation and high formation endotype?

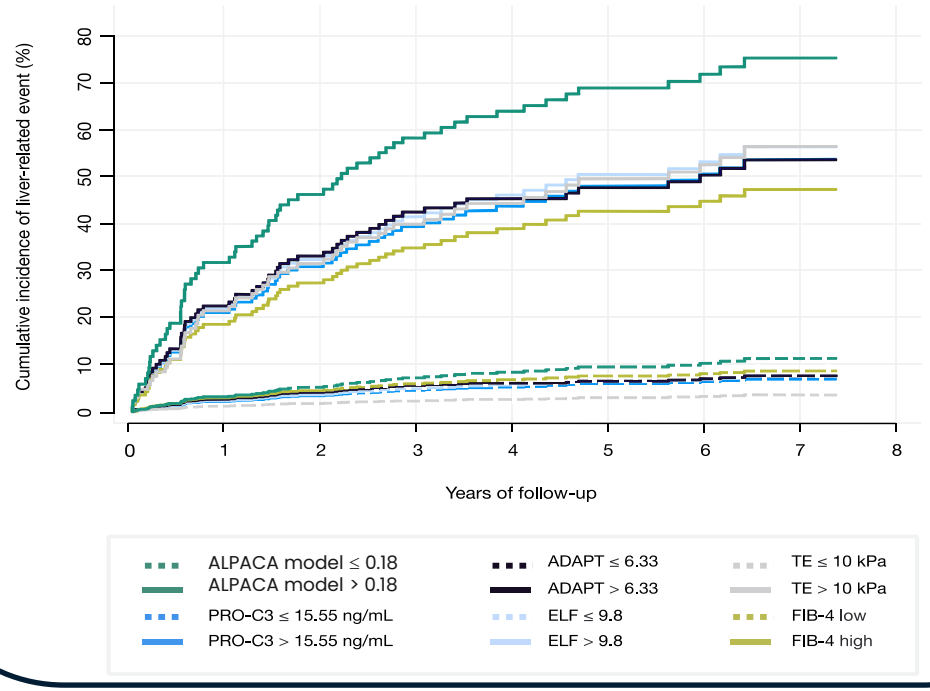


NORDIC BIOSCIENCE

The cumulative incidence of developing a liver-related event for ALD patients

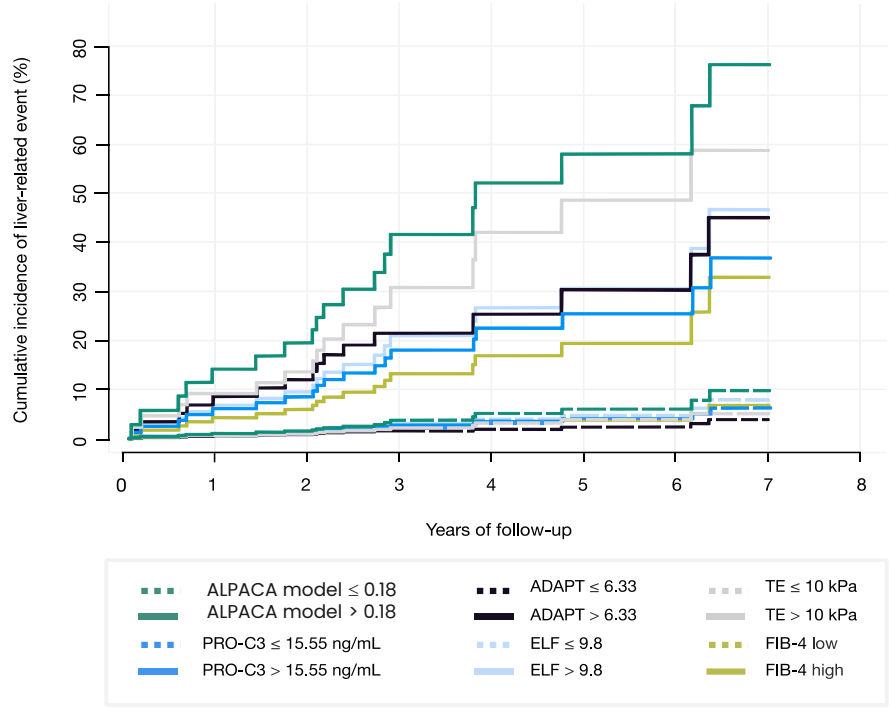
Derivation cohort – Secondary care

(n=222; F3/4: 42%)



Validation cohort – Primary care

(n=240; F3/4: 8%)



	Derivation cohort	Validation cohort
Patients, n	222	240
Liver-related events, n	65	19
All-cause mortality, n	56	21

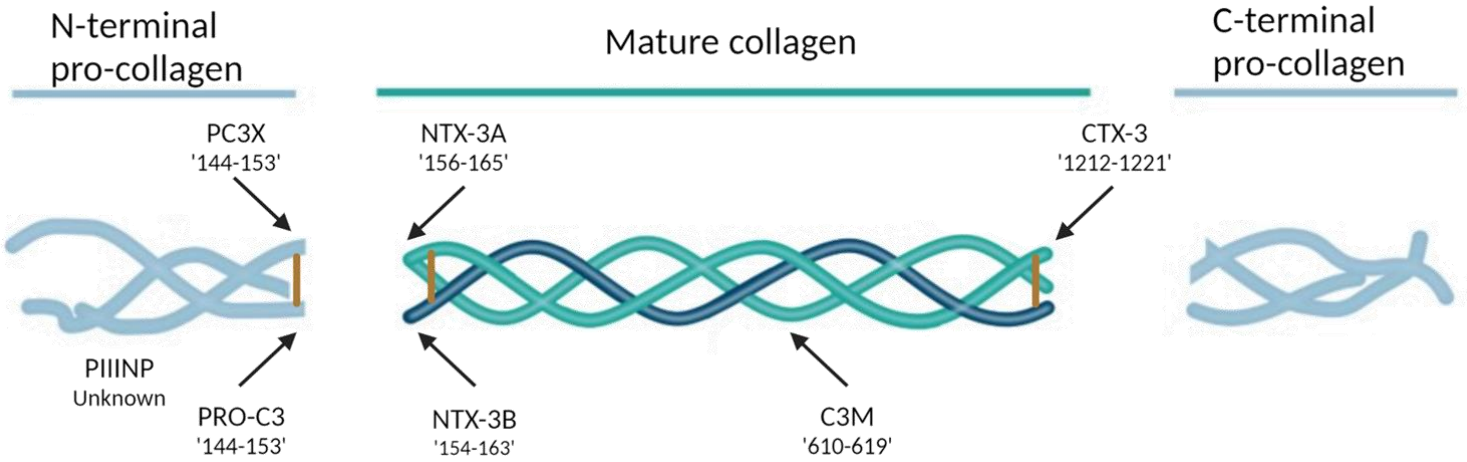
Johansen S et al; AASLD 2021

ALPACA model:
PRO-C3, AST/ALT, platelets

ADAPT:
PRO-C3, Age, T2DM, platelets

FIB-4:
Age, AST, ALT, platelets

The epitope matters – collagen is not just collagen

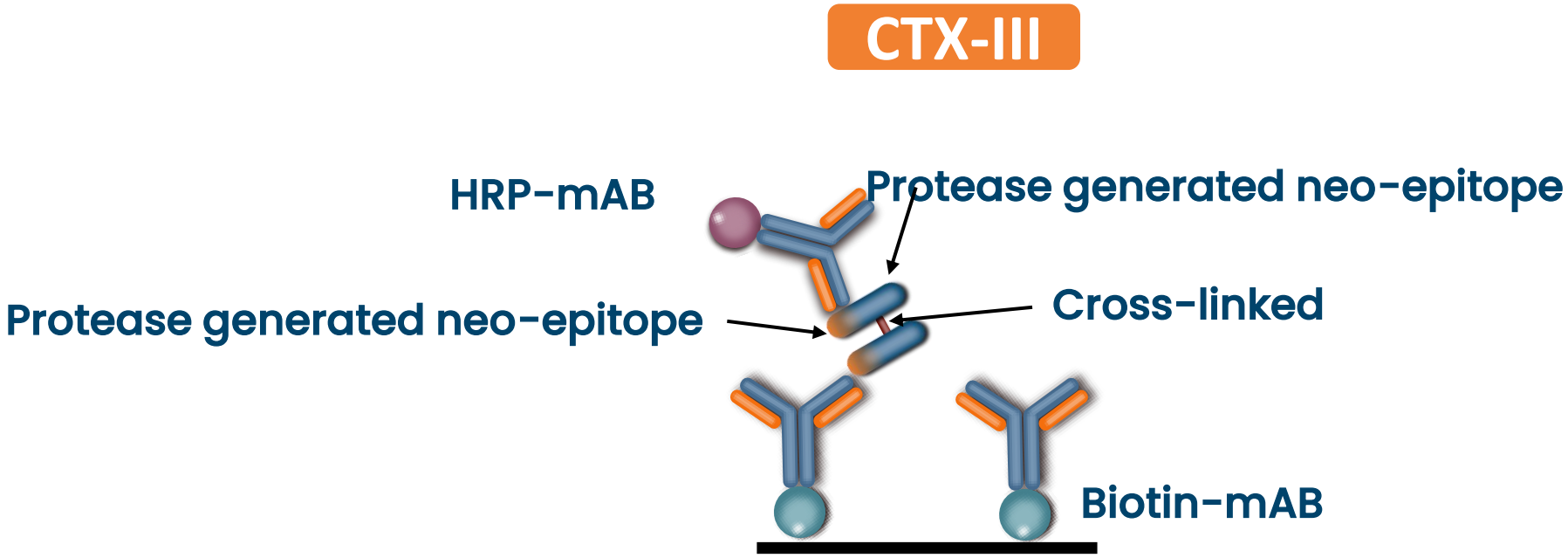


Name	Description	Number of clips to generate fragment	Process
P3NP	N-terminal pro-peptide of type III collagen	Unknown	Fibrosis formation (young collagen)
PRO-C3	N-terminal pro-peptide of type III collagen generated by ADAMTS-2	1	
PC3X	Cross-linked N-terminal pro-peptide of type III collagen generated by ADAMTS-2	2	Tissue stiffness
NTX-3A	N-terminal telopeptide fragment of cross-linked type III collagen generated by MMP3, -8, -9 and CatS	4	Fibrosis resolution (old collagen)
NTX-3B	N-terminal telopeptide fragment of cross-linked type III collagen generated by ADAMTS-2	4	
CTX-3	C-terminal telopeptide fragment of cross-linked type III collagen generated by BMP-1	4	
C3M	MMP-9 cleaved type III collagen	1	Inflammation

23

NORDIC BIOSCIENCE

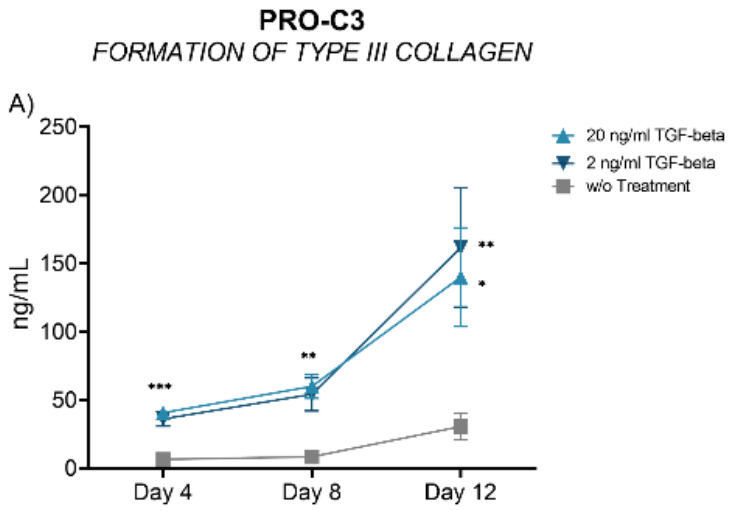
Fibrosis resolution - MMP degraded and cross-linked type III collagen - CTX-III

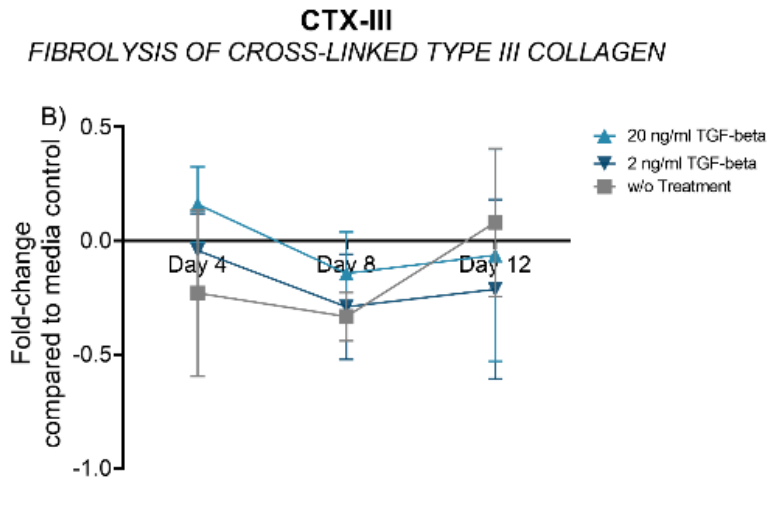


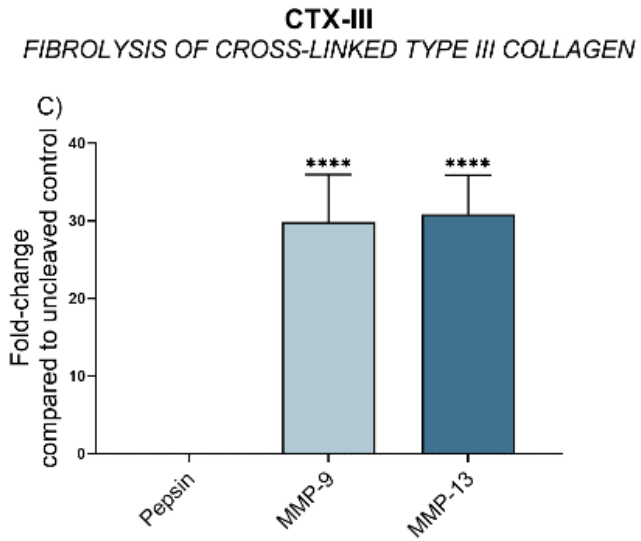
Pehrsson M, et al, 2022

Nordic Bioscience

CTX-III only comes from the degraded ECM

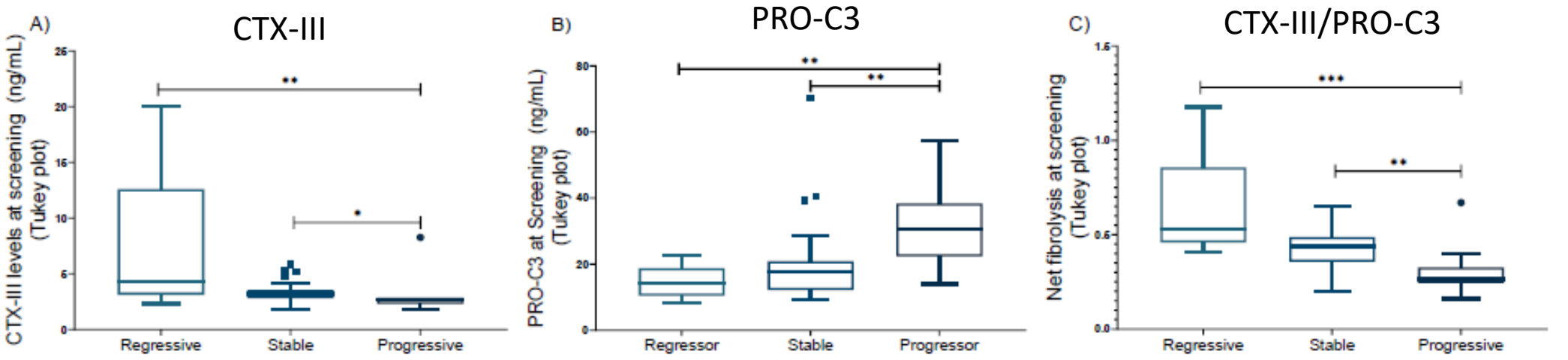






Pehrsson M, et al, 2022

A direct marker of fibrosis resolution



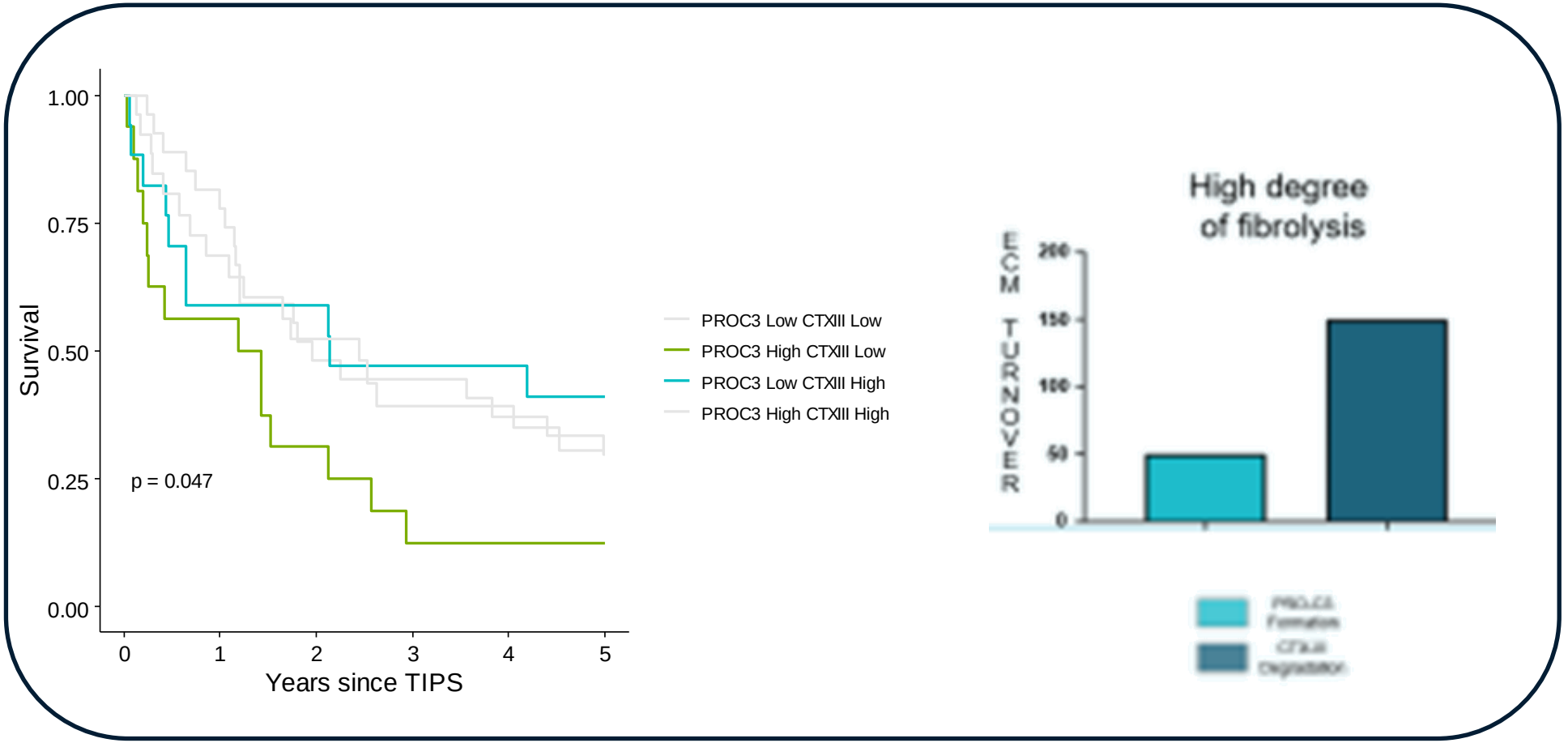
Patients were divided into three groups representing their fibrotic phenotype: Regressive, Stable, or Progressive. The respective levels of CTX-III (A), PRO-C3 (B), and net fibrolysis (C) was plotted for comparison. Significance depicted as: p<0.05, p<0.01**, and p<0.001****

Pehrsson M, et al, 2022

Statistical model: Kruskal-Wallis

N= 194 G/T1 HCV patients (NCT00244751), Ishak stage 2-4, HCV: hepatitis C virus. Liver biopsy week 0 and 56. Farglitazar vs pl treatment

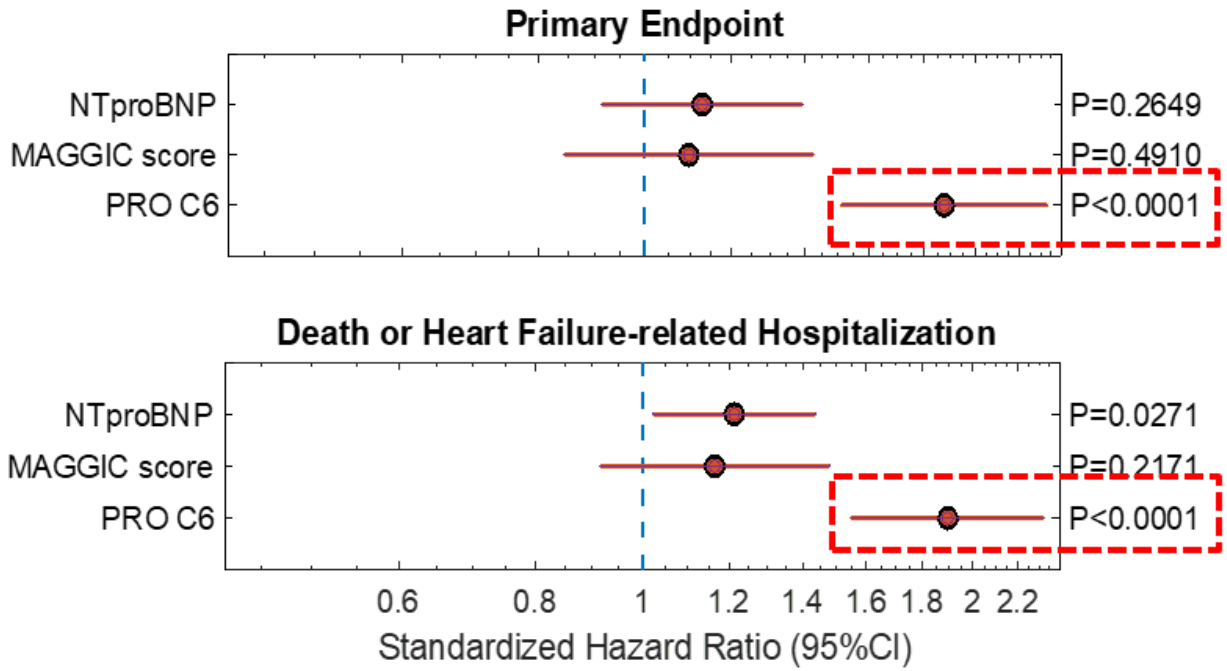
CTX-III and PRO-C3 related to survival in cirrhotic patients with TIPS

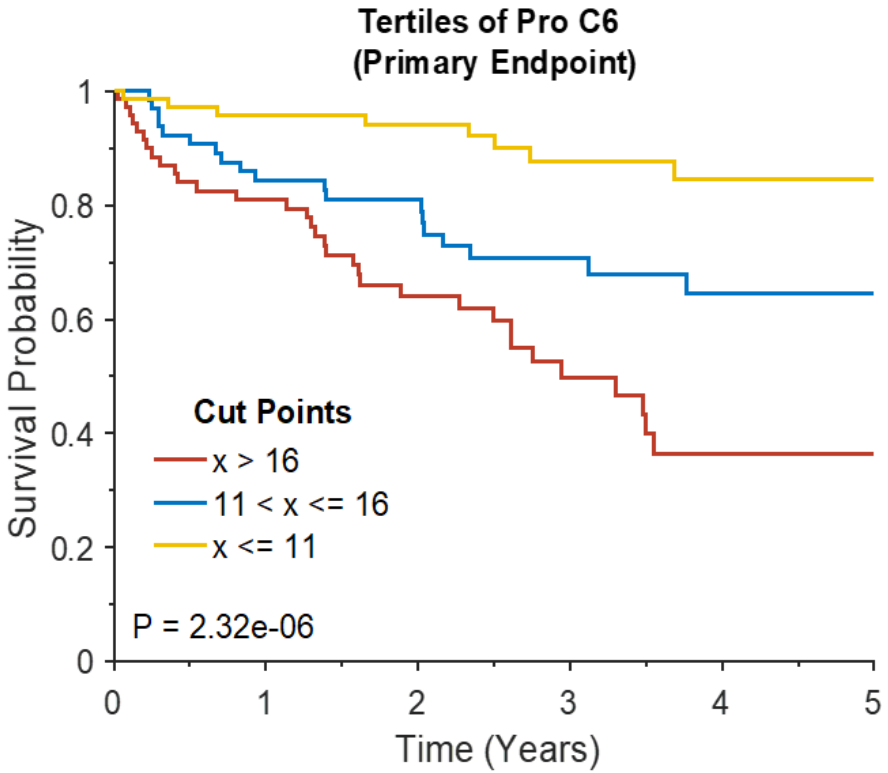


High degree of fibrolysis is associated with better prognosis

CVD (HFpEF) – fibroblasts proteins – collagens are dangerous

Results from the TOPCAT trial





Primary endpoint: CV death from CV causes, aborted cardiac arrest or hospitalization for HF

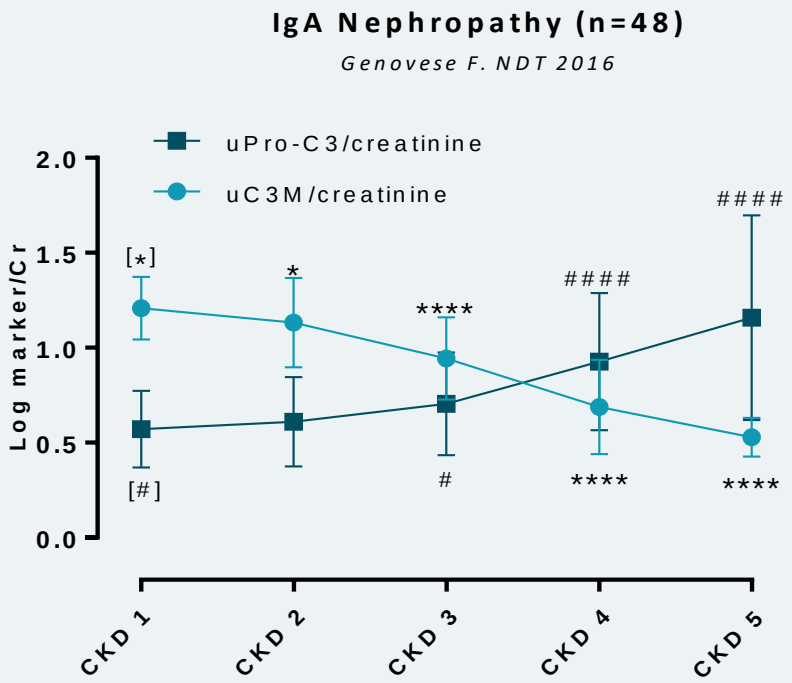
Data adjusted for age, gender, race, diabetes, history of MI & glomerular filtration rate (GFR)

Marker	Description
PRO-C6	Formation of collagen type VI and endotrophin

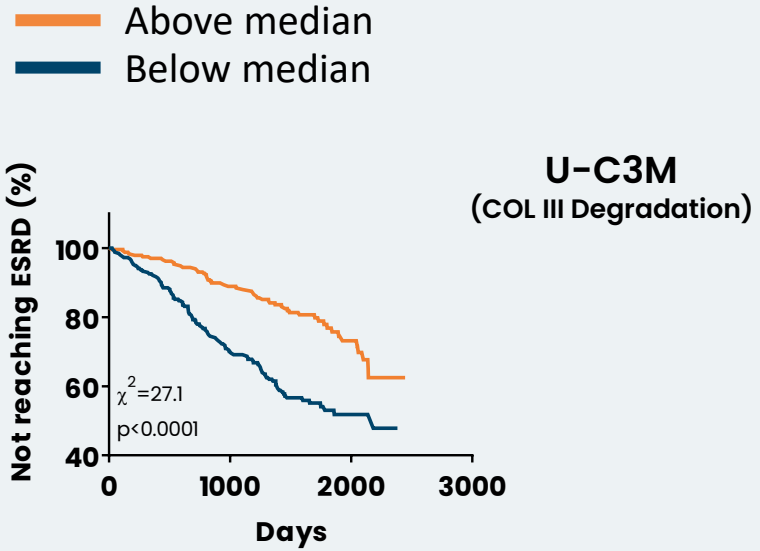
Chirinos et al. poster presentation at AHA 2019

The balance of COL III formation and degradation is changed in kidney disease

Understanding turnover

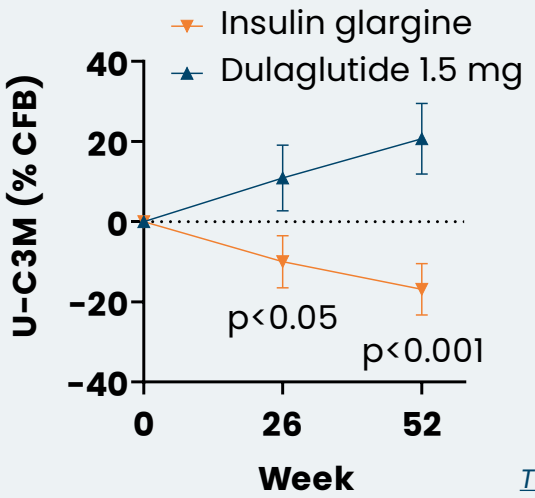


Prognostic



Genovese et al Clinical Kidney Journal, 2020

Pharmacodynamic



Tuttle et al Diabetes, 2020

Acknowledgements

Nordic Bioscience:

Diana J Leeming
Mette Juul Nielsen
Pernille Juhl
Ida Falk Villesen
Ida Christensen
Sarah Rønnow
Jannie M Sand
Martin Pehrsson
Rune Vestermark
Mayuur Bajaj
Sarah Wichmann
Morten Karsdal
Tina Manon-Jensen
Natasja Gudmann
Samuel Daniels
Peder Frederiksen
Kishwar Musa
Elisabeth Erhardtsen
Camilla Bramlev
Bafren Esmael
Susanne Hansen

Hepatology collaborators:

- Flemming Bendtsen, Søren Møller, Julie Pedersen, Liselotte Gluud, Hvidovre Hospital, DK
- **Aleksander Krag**, Maja Thiele, Stine Johansen, Mads Israelsen, Nikolaj Torp, Bjørn Madsen, OUH, DK
- Neil Guha, Guruprasad Aithal, Will Irving, Jane Grove, University of Nottingham, UK
- Keyur Patel, University of Toronto, CA
- **Quentin Anstee**, University of Newcastle, UK
- **Detlef Schuppan**, Jörn Schattenberg, University of Mainz, DE
- Jacob George, Mohammed Eslam, Westmead Hospital, AUS
- Takumi Kawaguchi, Kurume University, JP
- Leon Adams, University of Western Australia, AUS
- Karine Clement, Vlad Ratziu, Pierre Bel-Lassen, Pitié-Salpêtrière Hospital, FR
- Eric Lefebvre, Star Seyedkazemi, Laurent Fisher, Henrik Langren, Richard Torstensson, Pamela Vig, Andrew McKibben (CENTAUR, Tobira/Allergan)
- **Judith Ertle,** Corinna Schoelch, Boehringer Ingelheim, DE
- Diane Shevell, Yi Lou, Edgar Charles, Melissa Harris, Joel Myers, BMS, US
- Wladimiro Jimenez, University of Barcelona, ESP
- Arun Sanyal, Zachery Goodman, VCU Health, US
- Ken Cusi, Fernando Brill, University of Florida, US
- Rohit Loomba, Cyrielle Caussy, UCSD, UK
- Massimo Pinzani, Giuseppe Mazza, Douglas Thorburn, UCL, UK
- Mette Vesterhus, Tom Karlsen, University of Oslo, N