DEGRADATION OF THE ALVEOLAR BASEMENT MEMBRANE TYPE IV COLLAGEN ALPHA-3 CHAIN IS ASSOCIATED WITH ANTIFIBROTIC TREATMENT AND PULMONARY HYPERTENSION IN IDIOPATHIC PULMONARY FIBROSIS

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BACKGROUND

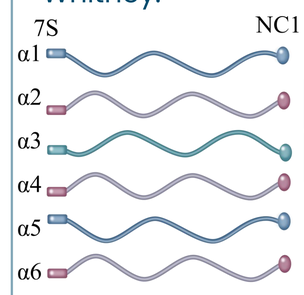
Idiopathic pulmonary fibrosis (IPF) is a rare but devastating disease with inevitable progression and high mortality. Currently, Pirfenidone and Nintedanib are the only approved antifibrotic treatment options to slow disease progression. Additionally, a pulmonary hypertension (PH) complication can further worsen disease outcome and quality of life. Forced vital capacity (FVC) is currently the most employed endpoint in clinical trials to monitor disease progression for antifibrotic treatment (Tx) development.

Molecularly, the extracellular matrix is subjected to excessive, pathological remodeling during IPF progression. Type IV collagen (COL4) is a critical part of the basement membrane that support the epithelium and crucial to cellular integrity. Mature COL4 are trimers that can be derived from six different alpha-chains, wherein the α3-chain is predominantly expressed in alveoli. Other trimers are expressed ubiquitously.

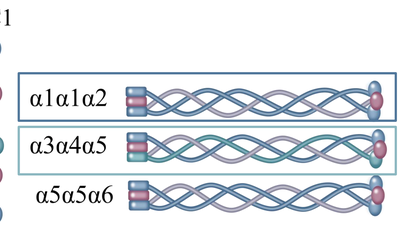
The aim was to investigate potential associations with antifibrotic Tx and PH in IPF patients by comparing serological levels of alveolar basement membrane degradation by a fragment of the COL4 alpha-3 chain (C4Ma3) with a fragment of more general remodelling by the alpha-1 chain (PRO-C4).

METHODS

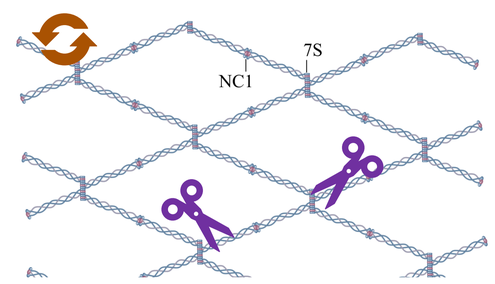
Serum C4Ma3 and PRO-C4 was assessed in 101 prevalent IPF subjects by the specific ELISA nordicC4Ma3™ and nordicPRO-C4™, utilizing a monoclonal antibody highly specific towards an epitope generated due to ECM degradation and remodeling. Clinical parameters were collected at time of blood sampling. Serum levels were compared in subjects by linear regression model adjusting for age and sex, spearman correlation test, and Mann-Whitney.



Type IV collagen monomer chains



Type IV collagen trimers



Mature type IV collagen quaternary structure undergoing remodelling (arrows) and proteolytic degradation (scissors)

Illustration adapted from Sand JMB, Madsen SF, Karsdal MA, 2024, Biochemistry of Collagens, Laminins, and Elastin, Chapter 4: Type IV collagen

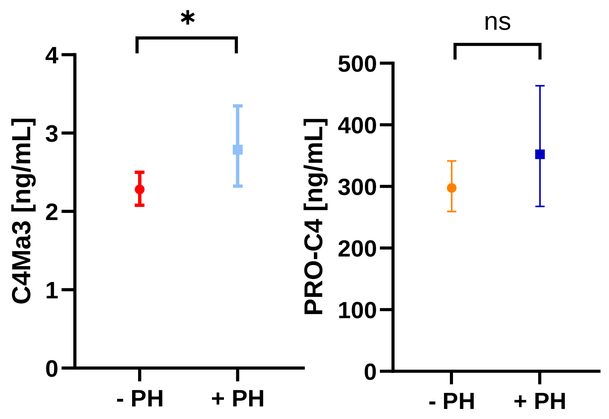
RESULTS

Serum C4Ma3 was significantly increased in IPF with PH, where serum PRO-C4 was not

C4Ma3 was higher in IPF with PH (+PH) (n=14, geometric mean 2.79 [95%CI: 2.32-3.35] ng/mL) compared to IPF patients without (n=87, geometric mean 2.28 [95%CI: 2.08-2.50 ng/mL; p=0.047).

PRO-C4 did not show significant differences in IPF with PH (n=14, geometric mean 352.1 [95%CI: 267.6-463.4] ng/mL) when compared to IPF patients without (n=87, geometric mean 297.6 [95%CI: 259.2-341.6] ng/mL, p=0.26)

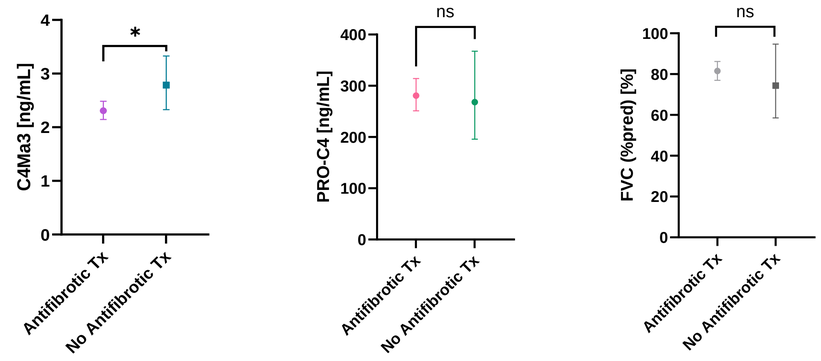
Data is shown as geometric mean (±95%CI).



Demographics	IPF
n	101
Age (years), median (IQR)	73 (68-76)
Male sex, n (%)	81 (80.2)
BMI, mean (SD)	26.2 (±4.0)
Smoking status, n (%)	
Never	29 (28.7)
Former	67 (66.3)
Current	5 (5.0)
Months since diagnosis, median (IQR)	11.8 (6.2-32.1)
FVC (% pred), mean (SD)	83.9 (±24.3)
DLCO (% pred), mean (SD)	46.1 (±14.4)
Antifibrotic Treatment, n (%)	
Pirfenidone or Nintedanib	83 (82.2)
No antifibrotic treatment	18 (17.8)

BMI, body mass index; DLCO, diffusing capacity for carbon monoxide;
FVC, forced vital capacity; SD, standard deviation;

Serum C4Ma3 was significantly decreased in IPF patients receiving antifibrotic treatment, where serum PRO-C4 was not

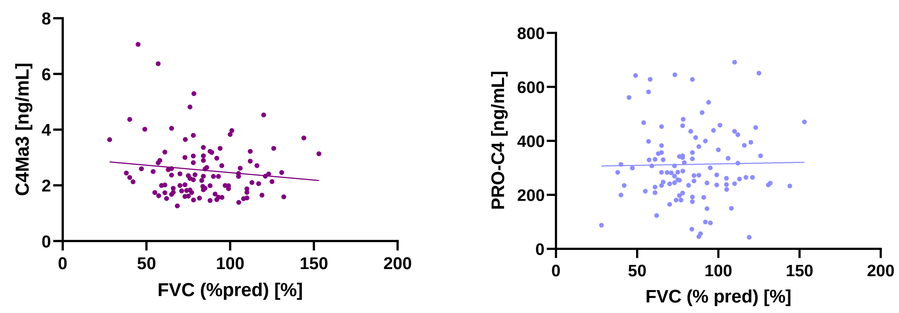


A potential pharmacodynamic effect was seen in those receiving antifibrotic treatment (Tx) with C4Ma3 being lower (geometric mean 2.31 [95%CI: 2.15-2.48] ng/mL) than those without (geometric mean 2.78 [95%CI: 2.33-3.33] ng/mL, p = 0.030) current antifibrotic Tx.

PRO-C4 did not show a significant difference in patients receiving antifibrotic Tx (geometric mean 280.9 [95%CI: 251.2-314.1] ng/mL) compared to those without (geometric mean 268.3 [95%CI: 196.0-367.4] ng/mL, p=0.80). The same applied when comparing FVC in these two groups; those receiving antifibrotic Tx (geometric mean 74.4 [95%CI: 58.5-94.7] % predicted) did not have statistically different FVC % predicted levels to those without (geometric mean 81.5 [95%CI 77.0-86.2] % predicted, p= 0.8)

Data is shown as geometric mean (±95%CI).

FVC was not associated with serum C4Ma3 or PRO-C4



Neither serum C4Ma3 (p=0.54) or PRO-C4 (p=0.95) was correlated with forced vital capacity (FVC).

Data is shown as a scatterplot with a linear regression line indicated.

Key messages

The COL4 alpha-3 chain has limited tissue distribution and is crucial for alveolar function. In this study, **higher** levels of COL4 alpha-3 chain degradation (**C4Ma3**) was:

- found in IPF with PH.
- associated with no antifibrotic treatment, indicating a pharmacodynamic potential.

In comparison, the COL4 alpha-1 chain marker **PRO-C4,** indicating ubiquitous basement membrane remodelling, did not show statistically different levels between any of the groups compared in this study. This could highlight the fact that damage done within alveoli are especially relevant when assessing the effect of antifibrotic Tx and PH in IPF.

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Disclosures: AHH, FBS, DJL, MAK, and JMBS are employed at Nordic Bioscience and may be shareholders.