Identifying biomarkers of fibrosis from the urinary proteome of renal fibrosis patients using DIA mass spectrometry

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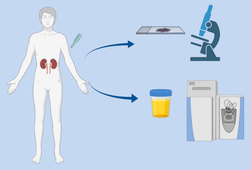
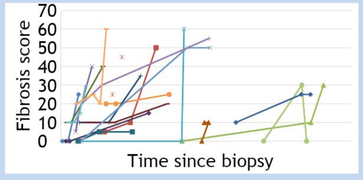
Background and objectives

Renal fibrosis is a hallmark of chronic kidney disease, entailing an excess accumulation of extracellular matrix components, primarily collagen species, disrupting organ architecture and leading to renal function loss. Histological assessment of renal tissue obtained by biopsy is the gold standard for determining the extent of fibrosis; however, the procedure is invasive. Urine is proximal to the site of disease activity and can be collected non-invasively, thereby making it an ideal matrix for biomarker discovery. Here, the results of a study to characterize the urinary proteome are presented focusing in particular on investigating collagen fragments as potential biomarkers for fibrosis in urine longitudinally sampled from renal transplant patients.

Cohort

Renal transplant patients (n=19) with 2-4 longitudinal biopsies with matched urine (n=56) were collected at Geneva University Hospital with informed consent.





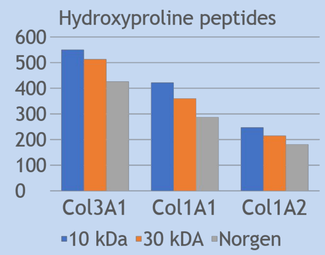
Methods

Urine samples were prepared with a 10 kDA filter-assisted preparation (FASP) followed by trypsinolysis and LC-MS/MS analysis. A spectral library was built from twelve pooled urine samples processed on a Q-Exactive-HF with a 120 min gradient using a Top 15 data-dependent acquisition (DDA) method. The resultant raw files were searched for both tryptic and semi-tryptic peptides using SpectroMine (Biognosys AG, Switzerland). Individual samples were processed using a data-independent acquisition method (DIA) with one full range MS1 and 22 DIA variable width windows and analyzed with Spectronaut. Biopsies were fixed and stained with trichrome stain and the extent of fibrosis was determined by a trained pathologist.

	Proteins	Peptides (with PTMs)
Renal fibrosis library (Tryptic & semi-tryptic)	3,481	54,114
Collagens	28	4,012
Collagen peptides with hydroxyproline		3,145
Collagen neoepitope peptides		3,657

Evaluation of three sample preparation methods: 10kDA FASP effectively captured collagen proteins & peptides

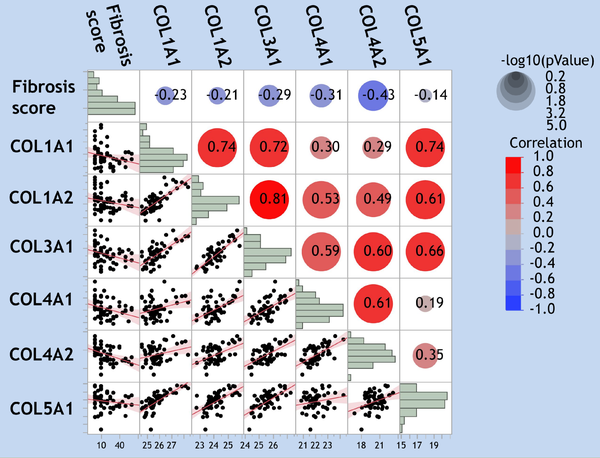
Preparation	Method description
1.	• FASP 10 kDa MWCO filters • Protein extraction
2.	• FASP 30 kDa MWCO filters • Protein extraction
3.	• Norgen ProteoSpin™ Urine Protein
All	• Tryptic digest • C18 cleanup for mass spectrometry



In this retrospective study, 56 urine samples from 19 renal transplant patients who had undergone >3 sequential kidney biopsies with progressive fibrosis score (0 to 60%) were analyzed by DIA.

	Proteins	Peptides	Collagen proteins	Collagen peptides
Average	1,888	21,301	18	2,120
Avg Lib Recovery (%)	54.2	52.9		
Total	3,034	49,580	22	3,596

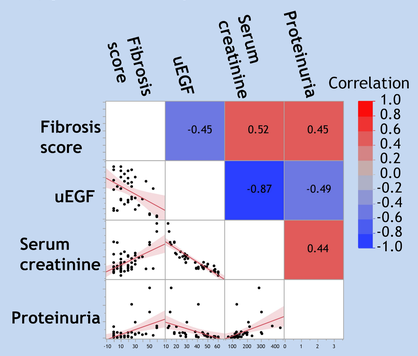
Fibrosis score moderately tracks with collagen protein family members, but interestingly several collagens were correlated with other collagens.



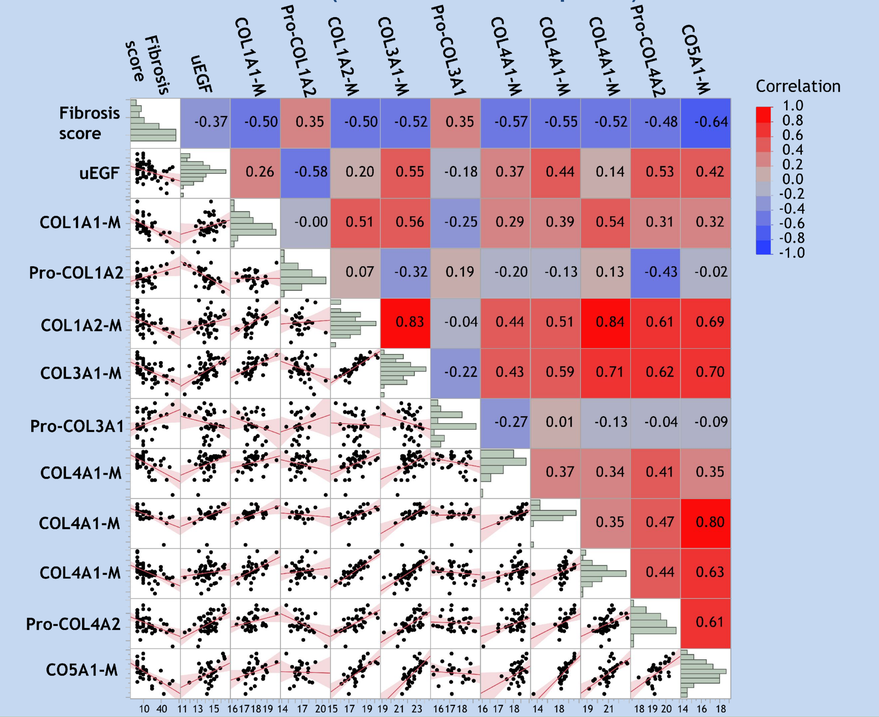
Results

It total, 29 peptides from nine different collagen proteins, were significantly correlated with fibrosis score (Pearson r >.4, p-value <.05) .

	Tryptic	Semi-tryptic	(Pearson r >.4, p-value <.05)
COL1A1	143	414	2
COL1A2	118	210	6
COL3A1	195	514	11
COL4A1	6	7	2
COL4A2	20	10	1
COL5A1	0	3	1
COL6A1	49	13	
CO12A1	34	7	1
COL15A1	13	3	1
COL18A1	20	12	2



Selected urinary collagen peptides from both the mature and pro-domains correlate with fibrosis score (Pearson correlation p<0.05).



Conclusions

Findings from this study suggest that changes in collagen peptides measured in urine may reflect ongoing fibrogenesis, degradation of existing fibrotic tissue, or tissue remodeling. This approach potentially offers a noninvasive view into fibrotic status and repeat sampling can be easily implemented in the context of a clinical trial. Urinary collagen peptides are being further investigated in independent cohorts, and results from this study will inform biomarker strategies in future clinical studies of novel antifibrotic agents.

References

Nat Immunol. 2019 Jul;20(7):902-914
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