A novel urinary marker of collagen type 1 degradation reflects kidney disease severity and fibrosis in IgA nephropathy.

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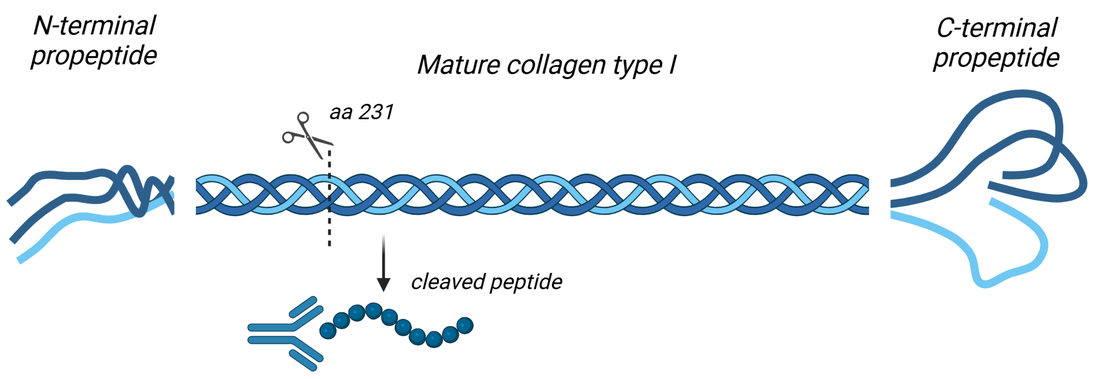
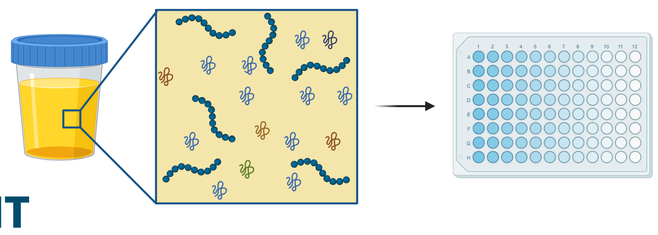
PURPOSE

In chronic kidney disease (CKD), **kidney fibrosis** is characterized by **increased collagen deposition and turnover**, especially of **collagen type I** (COL1), the primary protein in the kidney's extracellular matrix (ECM). Existing techniques for assessing kidney fibrosis are highly invasive and lack sensitivity, highlighting the need for a **non-invasive biomarker** to identify high-risk patients **before irreversible kidney function decline** occurs.



A **COL1 degradation peptide** (231_DDGEAGKPGP) was identified as **highly associated with kidney function decline** in urine peptidomics studies in CKD patients (3).

Aim of the study: To develop an immunoassay to detect this peptide in urine and evaluate its usefulness in individuals with Immunoglobulin A nephropathy (IgAN).



TECHNICAL ASSESSMENT

The N-terminal site of the DDGEAGKPGP peptide (**COL1A1_NEOC_231_abN**) was targeted using a highly specific monoclonal antibody in **competitive enzyme-linked immunosorbent assay (ELISA)** format. The conditions of the assay were determined, and technical evaluation established a **measurement range** of 4.08 – 1000 ng/ml and **IC50** of 54.2 ng/ml. The **intra- and inter-assay variation** (CV%) were deemed low at 7.0% and 11.6%, respectively. The **accuracy** of the measured analyte was also successfully evaluated.

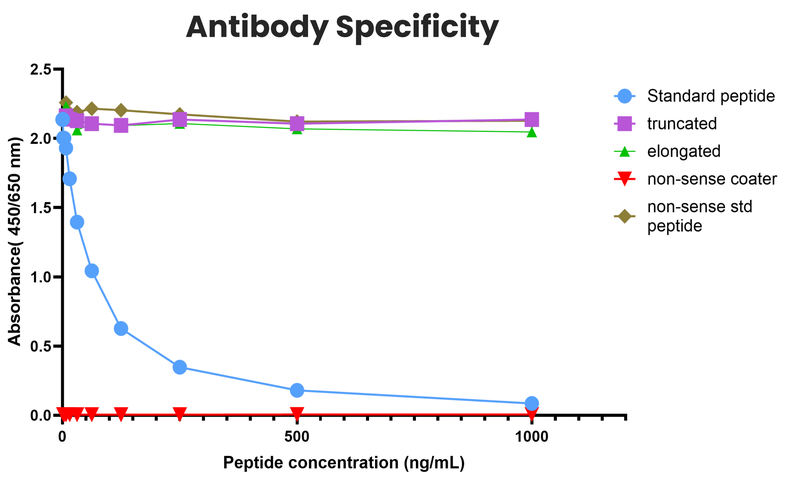


Figure 1: Specificity of the mAb produced for implementation into the immunoassay. The mAb is specific to the selection peptide (synthetically produced target sequence (DDGEAGKPGP)), shown by the high affinity of the mAb to this peptide and no affinity to the range of deselection peptides tested (elongated (GDDGEAGKPGP), truncated (DGEAGKPGP)).

CLINICAL COHORT – IgAN

The COL1A1_NEOC_231_abN concentrations were measured using ELISA in the urine of IgAN patients and healthy controls. The final biomarker concentration was adjusted for urinary creatinine (uCr) of the sample.

	IgAN (n = 54)	Controls (n=16)
Sex (male)	63%	38%
Age (years)	43.5 (median)	27.0 (median)
eGFR (ml/min/1.73m2)	46.1 (median)	-
Fibrosis score (%)	30.0 (median)	-

RESULTS

COL1A1_NEOC_231_abN biomarker is **reduced in IgAN compared to healthy** controls (p = 0.0007). The levels of COL1A1_NEOC_231_abN were also significantly **lower in more severe CKD stages** compared to early CKD stages.

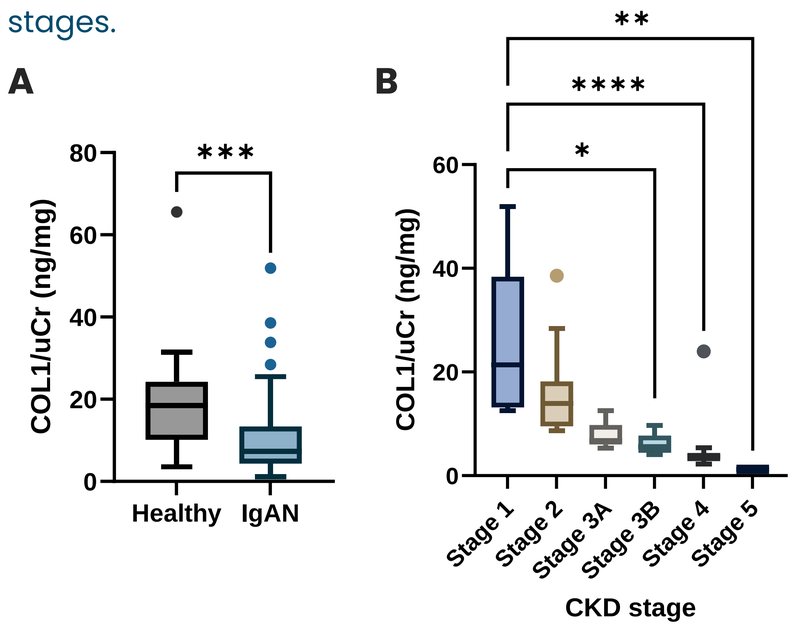


Figure 2: The COL1 biomarker (COL1A1_NEOC_231_abN) is lower in IgAN individuals compared to healthy controls (A), and the biomarker decreases further with increasing disease severity (CKD stage) (B). (A) Mann-Whitney test, p = 0.0007. (B) Stage 1 vs Stage 3B, p = 0.0142; Stage 1 vs Stage 4, p < 0.0001; Stage 1 vs Stage 5, p = 0.0018. All biomarker measurements are adjusted for uCr.

COL1A1_NEOC_231_abN is **highly correlated to eGFR**, a clinical measure for kidney function, and it is **inversely correlated to fibrosis score (%)** measured by histological staining and quantification.

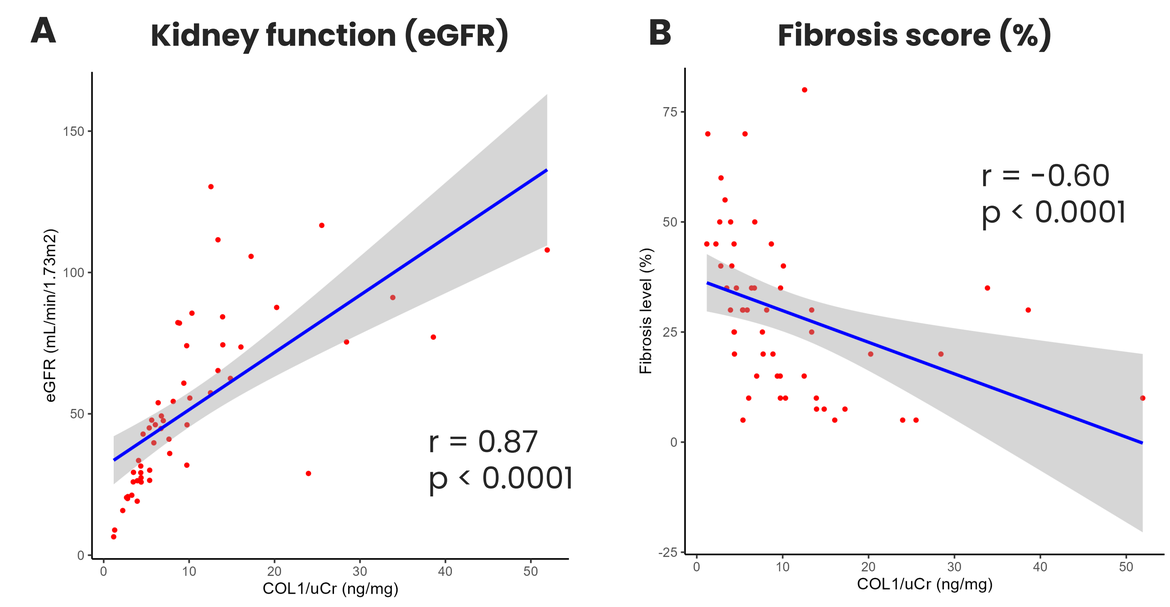


Figure 3: The COL1A1_NEOC_231_abN biomarker is positively correlated to kidney function represented by eGFR (ml/min/1.73m2) (A) and inversely correlated to fibrosis score (%) (B). Spearman's correlation: (A) r = 0.87, p < 0.0001, (B) r = -0.60, p < 0.0001. All biomarker measurements are adjusted for uCr.

CONCLUSION

We developed a novel and robust urinary assay which showed potential as a non-invasive biomarker of kidney disease severity and fibrosis in IgAN. It will be of interest to evaluate its prognostic potential in appropriate kidney disease cohorts and further in other organ diseases to understand the specificity of the biomarker to CKD.

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Figures created in Biorender, GraphPad Prism and R-Studio.

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