Clinical Kidney Journal, 2025, vol. 18, no. 11, sfaf280

https://doi.org/10.1093/ckj/sfaf280
Advance Access Publication Date: 23 October 2025
Original Article

ORIGINAL ARTICLE

Novel biomarkers of crescentic glomerulonephritis identified by urinary proteomics

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ABSTRACT

Introduction. Crescentic glomerulonephritis (CrGN) with rapidly progressive renal function loss necessitates prompt pathology diagnosis and treatment. Non-invasive biomarkers are crucial in cases where renal biopsy is unavailable or unsuitable. Urinary proteomics, particularly data-independent acquisition (DIA) proteomics, might provide potential indicators.

Methods. We recruited crescentic nephritis proved by renal biopsy at Peking Union Medical College Hospital (PUMCH) from May 2022 to April 2023 and age-matched nephritis, acute kidney injury (AKI), and health controls. The CrGN group is the patients with extensive glomerular crescents over 50%. We performed liquid chromatography with tandem mass spectrometry analysis to identify differentially expressed proteins (DEPs), ingenuity pathway analysis (IPA), and the proteome map for significant pathways and crucial proteins among patients and controls, then validated using enzyme-linked immunosorbent assay analysis.

Results. We enrolled a total of 137 participants, 55 in the proteomics cohort [15 CrGN (Type I: $n = 1$, II: $n = 3$, III: $n = 11$), 10 AKI, 15 non-crescentic nephritis, 15 healthy controls] and 82 in the validation cohort (33 CrGN, 6 AKI, 43 nephritis). Males occupied 42.3%, and the average age was 48 years of age. IPA analysis showed that neutrophil degranulation and complement cascade were the top two pathways in CrGN but not in the healthy and nephritis groups. Pathway analysis revealed activation of the neddylation pathway in CrGN compared to AKI patients. After integrating the DEPs among three groups via the Venn plot, we observed eight DEPs significantly associated with the CrGN, among which Coagulation factor V (F5), Phospholipid transfer protein (PLTP), and alcohol dehydrogenase 1C were significant proteins. The area under the curve values of F5 and PLTP in the validation cohort for predicting CrGN were 0.831 and 0.780 ($P < .001$).

Conclusion. By non-invasive urine proteomics, the new biomarkers F5/UCr and PLTP/UCr hold promise in identifying the CrGN patient.

Keywords: crescentic nephritis, data-independent acquisition proteomics (DIA), urine biomarkers

Received: 21.3.2025; accepted: 9.9.2025

KEY LEARNING POINTS

What was known:

- Crescentic glomerulonephritis (CrGN) is a rapidly progressive renal disease requiring timely diagnosis and treatment.
- Traditional diagnostic methods, such as renal biopsy, are invasive and carry risks.
- Non-invasive biomarkers for detecting disease activity and guiding treatment decisions were lacking.

This study adds:

- This study identifies urinary Coagulation factor V (F5) and phospholipid transfer protein (PLTP) as novel biomarkers for CrGN using data-independent acquisition (DIA) proteomics.
- Pathway analysis reveals the activation of neutrophil degranulation, complement cascade, and neddylation pathways as key mechanisms in CrGN.

Potential impact:

- Urinary F5 and PLTP biomarkers may offer a non-invasive, clinically useful tool for diagnosing and monitoring CrGN.
- The pathway insights could guide future therapeutic strategies targeting complement and neutrophil activation pathways, potentially improving patient outcomes.

INTRODUCTION

Crescentic glomerulonephritis (CrGN), which usually presents as AKI or even progressive GN, is one of the most severe causes of rapid renal function loss. The primary causes of CrGN are so complicated and include immune-complex GN, anti-glomerular basement membrane GN (anti-GBM diseases), ANCA-associated vasculitis (AAV), C3 glomerulopathy, and monoclonal immunoglobulin-associated renal diseases, among others [1]. Multiple causes and varied clinical characteristics pose significant challenges in the differential diagnosis of CrGN and depend on the pathological evidence of renal biopsy in clinical practice. Unfortunately, the patient's participation, acceptance, and relative contraindications always limit the ability to execute prompt renal puncture biopsy, affecting the diagnosis and treatment of these patients who bear a risk of a poor renal prognosis. Furthermore, retrospective biopsy-based series have reported a prevalence varying from 2.1% to 4.2% [2, 3]. Therefore, non-invasive biomarkers and predictive models for CrGN are urgently needed to assist with timely and accurate individualized diagnosis and treatment.

Given the urgent clinical needs, many scholars have explored the biomarker and prediction models, such as urinary iron death inhibitory protein (FSP1) from AKI and compatible sensitivity and specificity in CrGN [4]. Recent studies have demonstrated that proteomics contributed to screening a panel of new biomarkers of activity and prognostic for renal disease [5, 6]. However, there is still a shortage of biomarkers focused on the general mechanism of crescent formation for different reasons, with urgent need for further in-depth studies. Therefore, this study intends to apply DIA proteomics technology to discover biomarkers associated with CrGN for early clinical identification and medical treatment.

MATERIALS AND METHODS

Baseline clinical characteristics of proteomics cohort

This prospective study utilized consecutively collected urine samples from patients undergoing renal biopsy at Peking Union Medical College Hospital (PUMCH) between May 2022 and April 2023. We recruited age-matched healthy controls and divided the patients into the CrGN, nephritis, and non-crescentic AKI group (Fig. 1). The CrGN group was patients with a pathologic condition characterized by extra capillary proliferation in >50% of glomeruli. The CrGN group was classified into three types, including Type I (anti-glomerular basement membrane antibody disease), Type II (immune-complex mediated), and Type III (pauci-immune, typically AAV) [1]. Classification was based on immunofluorescence, serological testing, and renal biopsy findings. The screening criteria for the nephritis group were GN without crescent formation. AKI group was patients with a rise in SCr of ≥26.5 mol/l within 48 h, or in SCr to ≥1.5 times the baseline value within 7 d, or a urine output of <0.5 ml/kg/h for six consecutive hours, and diagnosed as AKI by renal pathology with no crescentic nephritis. Healthy controls were recruited from individuals undergoing routine health check-ups at PUMCH who provided informed consent. Inclusion criteria required normal serum creatinine and estimated glomerular filtration rate (eGFR ≥90 ml/min/1.73 m²), along with unremarkable urinalysis results, including absence of hematuria, proteinuria, and leukocyturia. Additionally, participants were screened through medical history review and physical examination to exclude known risk factors for kidney disease. Composite adverse renal outcomes were defined as progression to end-stage renal disease (ESRD), requirement for dialysis, or a sustained ≥50% decline in eGFR from baseline. Baseline clinical characteristics were collected and documented with the blood and urine examinations on admission. The Ethical Committee approved the PUMCH study (No. K2185). Informed consent is taken from all the participants present in the study.

LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY ANALYSIS

Mid-stream urine samples were collected in the morning and immediately centrifuged at 4500 rpm for 15 minutes at 4°C to remove cellular components and debris, then collected the supernatant and stored at −80°C for further experiments. Later, the urine samples were processed using a urine proteome preparation kit (cat. no. PN-23677, Biomsomics). One microgram of the samples was analyzed using an analytical column (Thermo Scientific, 75 μm × 250 mm, 2 μm) on a Vanquish connected to an Orbitrap Exploris480 mass spectrometer (Thermo Scientific). We eluted the peptides by a binary solvent system with 99.9% H_2O, 0.1% formic acid (phase A), and 80% ACN, 19.9% H_2O, 0.1% formic acid (phase B). The following linear gradient was used: 0%–1% B in 5 min, 1%–6% B in 1 min, 6%–23% B in 66 min, 23%–35% B in 6 min, 35%–90% B in 2 min, 90%–100% B in 1.5 min,

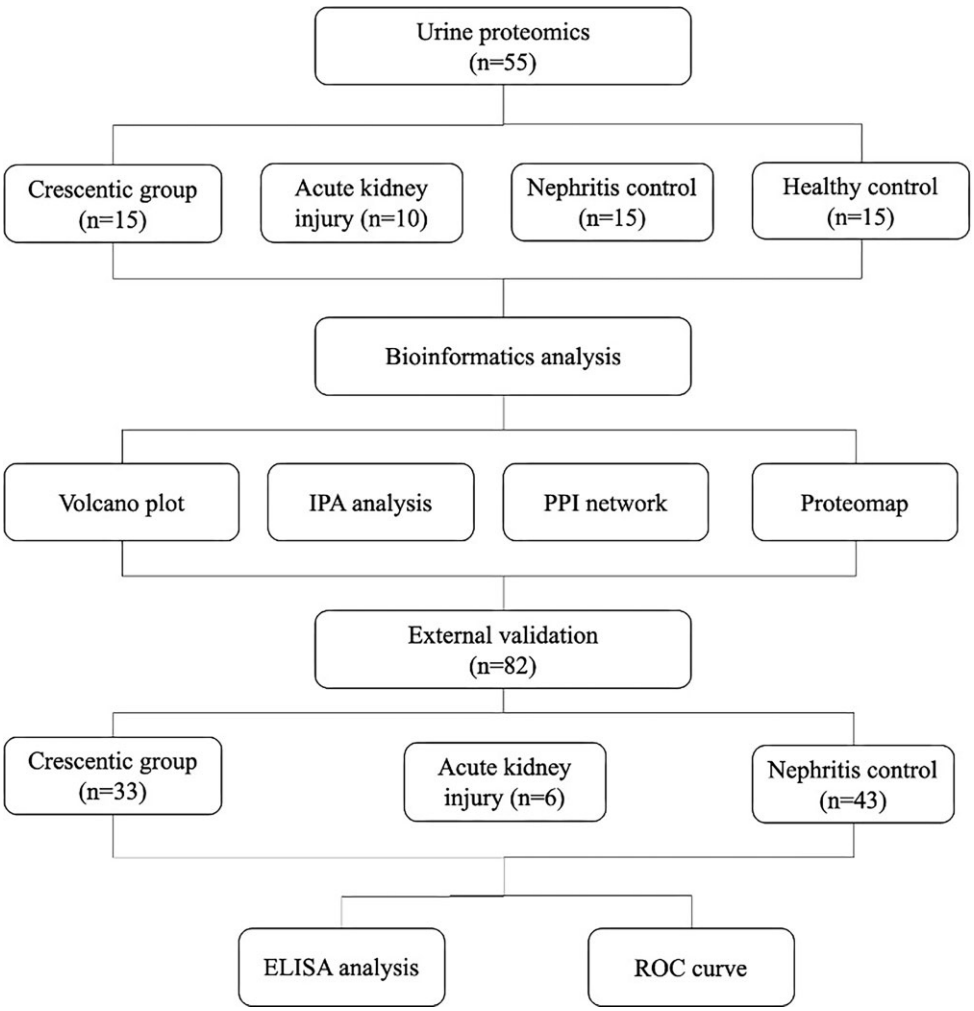


Figure 1: Patient flowchart. Flow diagram of participant enrollment and grouping. A total of 137 participants were recruited: 55 in the proteomics cohort (15 CrGN, 10 acute AKI, 15 non-crescentic nephritis, 15 healthy controls), and 82 in the validation cohort (33 CrGN, 6 AKI, 43 nephritis). CrGN was defined by >50% glomerular crescents on renal biopsy. AKI controls had non-glomerular AKI without crescents. Exclusion criteria included inadequate samples or incomplete clinical data. PPI, protein–protein interactions network.

and equilibrated with 100% A for 0.5 min. The eluent was introduced directly to an Orbitrap Exploris480 mass spectrometer via an EASY-Spray ion source. Source ionization parameters were: spray voltage, 2.3 kV and capillary temperature, 320°C. For the urine samples, a DIA MS method in which one full scan (350 to 1200 *m/z*, resolution 120 000 at 200 *m/z*) at a target of 3e6 ions was first performed, followed by 80 windows with a resolution of 30 000 where precursor ions fragmented with higher-energy collisional dissociation (stepped collision energy 25%, 30%, 35%) and analyzed with an AGC target of 2e5 ions and maximum injection time at 50 ms in mode using centroid positive polarity.

Data processing

We processed the raw data by DIA-NN v.1.8.1, then set the Enzyme specificity as C-terminal to arginine and lysine, with a maximum of two missed cleavages in the database search. Later, identified the peptide with an allowed initial precursor mass deviation of up to 10 ppm and an allowed fragment mass deviation of 0.02 Da, searched criteria including carbamidomethylation of cysteine as a fixed modification, and oxidation of methionine and acetyl (protein N terminus) as variable modifications. false discovery rate (FDR) was set to 1% at both protein and peptide precursor levels, with the search for library-free with *in silico* digestion, deep learning-based spectra, and retention time prediction. The human fasta file was from UniProt (2023 release, 81 803 entries).

DIA proteomics data analysis

For proteomic analysis of the collected clinical samples, proteins were filtered to retain those with at least 50% valid values across all samples, a commonly accepted threshold in proteomics data analysis [7]. Proteins with >50% missingness were excluded from downstream statistical analysis. Missing values in the remaining proteins were imputed using the *K*-nearest neighbor method, which estimates missing values based on sample similarity [8]. This imputation strategy, followed by median normalization, helps preserve data structure and reduce inter-experimental bias.

All statistical analyses and visualizations were performed using R software (version 4.3.2). Proteomic differential expression analysis was conducted using Perseus software, applying

Table 1: The clinical characteristics of proteomics cohort.

Variable	Total ($n = 55$)	CrGN group ($n = 15$)	AKI group ($n = 10$)	Nephritis group ($n = 15$)	Health group ($n = 15$)	P value
Male, *n* (%)	25 (45.5)	7 (46.7)	5 (50.0)	6 (40.0)	7 (46.7)	.964
Age (years)	52.8 ± 13.2	53.6 ± 14.5	49.3 ± 15.2	49.3 ± 15.0	57.9 ± 6.1	.259
Scr (μmol/l)	121.0 (75.0- 234.0)	235.0 (165.0- 550.0)[a]	284.5 (198.8–485.5)[a]	92.0 (83.0- 119.5)	71.0 (65.0–75.5)	<.001
eGFR (ml/min/ 1.73 m^2)	46.36 (23.52, 78.18)	23.70 (8.24,29.99)	17.01 (10.66,24.38)	62.86 (48.25,71.88)	88.37 (77.57,96.54)	<.001
24h-Pro (g)	0.60 (0.00–1.57)	1.41 (0.69–3.73)	0.91 (0.22–2.24)	0.74 (0.38–1.42)	NA	.210
CRP (mg/l)	0.96 (0.00–5.33)	2.00 (0.61–36.20)	2.17 (1.02–23.09)	1.94 (0.85–5.33)	NA	.881
ESR (mm/h)	17.0 (0.0–44.5)	63.5 (40.3–91.5)[a]	37.0 (36.0–40.0)[a]	17.0 (14.0–29.5)	NA	.031
D-dimer (mg/l)	0.35 (0.00–1.23)	1.46 (1.15–3.88)[a]	0.64 (0.21–1.23)[a]	0.40 (0.23–0.59)	NA	.003

24h-Pro, 24 h urine protein.
[a]CrGN group versus AKI group, SCr $P = .868$; ESR $P = .240$; D-dimer $P = .021$.

two-sample t-tests to compare groups. To identify differentially expressed proteins (DEPs), we applied thresholds of |log2 fold change| > 1 and area under the curve (AUC) > 0.8, criteria supported by prior proteomic studies [9, 10], to ensure both sensitivity and specificity. Statistical significance was determined using the Benjamini–Hochberg procedure to control the FDR < 0.01. For the final integrated analysis, we applied both criteria (FDR < 0.01 and AUC > 0.8) to identify DEPs with both high statistical significance and diagnostic value, and only proteins with FDR-adjusted *P* values <0.01 were retained as DEPs. Volcano plots for the quantified proteins expressed significantly differently among groups were created using the R package ggplot2. Protein enrichment pathways were analyzed using ingenuity pathway analysis (IPA), based on the Ingenuity Knowledge Base, biological interaction, and functional annotation database. A global protein interactome network for the proteins expressed was differentially built among groups using Cytoscape, and the protein–protein interactions were retrieved from the STRING database.

VALIDATION OF THE POTENTIAL BIOMARKER

We further validated the potential biomarker using enzyme-linked immunosorbent assay (ELISA) analysis in urine specimens in the validation cohort. The validation cohort was also derived from the same prospectively enrolled population. ELISA kits Coagulation factor V (F5, ab137976), Phospholipid transfer protein (PLTP, ab289907), and alcohol dehydrogenase 1C (ADH1C, AE23955HU) were from Abcam and Abebio. Hub gene expression levels were assessed by GraphPad Prism v.6.0, with the receiver operating characteristic (ROC) plotted to indicate the levels of hub genes distinguishing the crescentic nephritis.

Statistical analyses

Continuous variables were expressed as mean ± standard deviation for normally distributed data and as median with interquartile range for nonnormally distributed data. Categorical variables were presented as frequencies with percentages. Data distribution was assessed using the Shapiro–Wilk test and visual inspection of histograms. The Student's *t*-test and the Wilcoxon rank sum test were used to analyze continuous variable differences among groups. For predictive modeling, we applied a multilayer perceptron neural network, a feedforward artificial neural network architecture commonly used for classification tasks. The network consisted of one hidden layer and was trained using backpropagation with a learning rate of 0.1 and early stopping to prevent overfitting. The combined dataset from the proteomics and validation cohorts was randomly split into a training set (70%) and a validation set (30%). In the validation set, the model's predictive validity was evaluated by the ROC curves and mixing matrices. Cox proportional hazards models were used to assess predictors of composite adverse renal outcomes, with variables selected based on clinical or univariate significance ($P < .05$). All the statistical analyses were conducted with the R statistical software (version 3.4.3) and SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA).

RESULT

Clinical characteristics of patients in the proteomics cohort

Among 137 participants, 55 were in the proteomics study with half male and an average age of 53 ± 13 years (Table 1, Supplementary Table S1). The CrGN group contained Type I ($n = 1$), Type II ($n = 3$), and Type III ($n = 11$). AKI group contained acute interstitial nephritis (AIN, $n = 5$), acute-on-chronic kidney disease ($n = 4$), and LN ($n = 1$). The nephritis group was IgA-C0 (no crescents, $n = 15$). AKI group had the highest serum creatinine (SCr, $P < .001$), and the CrGN group had the highest erythrocyte sedimentation rate (ESR, $P = .031$), and D-dimer level ($P = .003$), but with no statistical difference between crescentic and AKI group in terms of SCr and ESR level.

We recruited 82 patients for the validation study (Table 2) with an average follow-up time of 11.8 ± 14.0 months. The CrGN group contained Type I ($n = 2$), Type II ($n = 18$), and Type III ($n = 13$). AKI group contained AIN ($n = 3$) and acute tubular necrosis ($n = 3$). The nephritis group contained IgA ($n = 18$), membranous nephropathy ($n = 11$), chronic interstitial nephritis ($n = 8$), minimal change disease ($n = 3$), and LN ($n = 3$). The CrGN group had the highest SCr and lowest eGFR among the three groups ($P < .001$), but there was no statistical difference between the CrGN group and the AKI group ($P = .077$, $P = .171$). Patients with AKI had the highest C-reactive protein (CRP) and ESR among the three groups ($P < .001$) without a significant difference between the crescentic and AKI groups. The patients in the CrGN group had the highest D-dimer level ($P < .001$) and the worst composite adverse renal outcomes, especially dialysis ($P < .001$).

Identification of DEPs in AAV and bioinformatics analysis

Urine proteomic analysis was performed using the DIA method to identify potential biomarkers and activated pathways of crescentic nephritis. Liquid chromatography with tandem mass spectrometry analysis identified 5561 proteins (specific peptides

Table 2: The clinical characteristics of validation cohort.

Variables	Total ($n = 82$)	CrGN group ($n = 33$)	AKI group ($n = 6$)	Nephritis group ($n = 43$)	P value
Male, n (%)	33 (40.24)	9 (27.27)	2 (33.33)	22 (51.16)	.122
Age (years)	44.1 ± 16.2	40.5 ± 18.7	60.3 ± 13.2	44.6 ± 13.0	.019
Hemoglobin (g/l)	116.0 (99.3, 137.0)	99.0 (87.0 107.0)[a]	113.5 (105.3 115.0)[a]	135.0 (120.5 145.0)	<.001
Albumin (g/l)	34.0 (29.3, 38.0)	31.0 (26.0,33.0)[a]	35.0 (34.0,38.3)[a]	37.0 (32.5,40.0)	<.001
Scr (μmol/l)	149 (73, 259)	281 (198, 430)[a]	229 (145, 244)[a]	74 (61, 125)	<.001
eGFR (ml/min/1.73 m^2)	38.7 (19.0 , 86.9)	18.6 (11.4,27.2)[a]	25.5 (19.2,35.1)[a]	81.7 (49.2 115.0)	<.001
CRP (mg/l)	2.33 (0.75, 13.28)	5.67 (1.52,22.30)[a]	14.52 (7.46,18.17)[a]	0.78 (0.39,3.34)	<.001
ESR (mm/h)	30.0 (11.8, 75.0)	63.0 (29.0,85.5)[a]	81.0 (67.5,94.5)[a]	14.0 (6.3,26.0)	<.001
24h-Pro (g)	2.24 (0.96, 4.44)	3.83 (1.81,6.74)[a]	0.78 (0.46,3.53)[a]	1.60 (0.76,3.30)	.003
U-RBC (cells/μl)	60 (11, 341)	323 (44, 826)[a]	19 (2, 38)[a]	33 (4, 91)	<.001
D-dimer (mg/l)	0.71 (0.26, 2.60)	3.37 (1.25,4.96)[a]	0.69 (0.42,2.09)[a]	0.33 (0.17,0.71)	<.001
Follow-up time (months)	11.8 ± 14.0	20.4 ± 18.5	6.7 ± 3.1	5.9 ± 4.2	<.001
Composite adverse renal outcomes	17 (20.73%)	14 (42.42%)	0 (0.00)	3 (6.98%)	<.001
Dialysis	12 (13.42%)	11 (33.33%)	0 (0.00)	0 (0.00)	<.001
Death	1 (1.22%)	1 (3.03%)	0 (0.00)	0 (0.00)	.382
Worsening renal function	12 (14.63%)	9 (27.27%)	0 (0.00)	3 (6.98%)	.037

24h-Pro, 24 h urine protein.

[a]CrGN group vs. AKI group, hemoglobin $P = .087$; albumin $P = .034$; SCr $P = .077$; eGFR $P = .171$; CRP $P = .309$; ESR $P = .269$; 24h-Pro $P = .064$; U-RBC $P = .028$; D-dimer $P = .028$.

P values represent overall group differences by Kruskal–Wallis test; pairwise comparisons are annotated with a.

≥1), of which 2578 were co-quantified proteins, with the screening condition that at least two of the quantification values.

The heatmap suggested significant differences between the CrGN group and health control, the CrGN group and nephritis control, and the CrGN group and AKI (Fig. 2, Supplementary Fig. S1). We identified 944, 410, and 129 DEPs (FDR < 0.01) in the CrGN group compared with health control, nephritis control, and AKI, respectively. After applying additional diagnostic relevance criteria (AUC > 0.8), 464, 200, and 40 DEPs remained for these comparisons, respectively. IPA pathway showed that neutrophil degranulation and complement cascade were the high-ranked pathways in the CrGN group, compared with health control and nephritis (Fig. 3, Supplementary Fig. S2, Fig. S3). Degradation of beta-catenin by the destruction complex, salvage pathway of pyrimidine ribonucleotides, and neddylation were signaling pathways in the CrGN group, compared with the AKI group. The protein–protein interaction network of DEPs indicated in the CrGN group with the activation of complement and coagulation cascades and neutrophil degranulation (Supplementary Fig. S4) compared to the nephritis group, and the activation of NF-kappaB and neddylation in B cells compared to the AKI group.

Using hierarchical proteomaps based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [11], we visualized functionally distinct categories within the DEPs (Fig. 4). Every tile (small polygon) represents one type of protein. Tiles are arranged and colored according to the hierarchical KEGG pathway maps. Upregulated proteins were highly involved in complement and coagulation cascades, glycan metabolism, and ubiquitin labeling. The overlap of the DEPs among the CrGN group, nephritis group, and AKI group led to the identification of eight hub DEPs (Fig. 5, Supplementary Table S2). After reducing the DEP screening criterion to |logFC|>1 and an AUC value of >0.8 for predicting crescentic nephritis, F5, PLTP, and ADH1C were selected as potential signature protein.

Diagnostic efficacy of the urine biomarker

We evaluated the diagnostic efficiency of the urine biomarker using ELISA analysis in the validation cohort. The CrGN group had the highest urinary PTLP/urine creatinine (UCr) and F5/UCr, without statistical significance in ADH1C/UCr (Fig. 6). Subgroup analysis stratified by CrGN type showed that compared with Type III, Type II had higher PLTP/UCr levels (6249.03 ± 1815.54 vs. 1781.65 ± 482.15 pg/mmol, $P = .028$) and ADH1C/UCr (129.96 ± 41.52 vs. 35.26 ± 8.06, $P = .038$ ng/mmol), while no statistical difference in F5/UCr levels ($P = .398$). Scatter plots revealed significant positive correlations between crescentic percentage and urinary PLTP/UCr ($r = 0.36$, $P < .001$), F5/UCr ($r = 0.40$, $P < .001$), and SCr ($r = 0.60$, $P < .001$) (Supplementary Fig. S5). The AUC values PLTP/UCr and F5/UCr for predicting CrGN were 0.831 (0.738–0.924, $P < .001$) and 0.780 (0.679–0.881, $P < .001$). Combining PLTP/UCr and F5/UCr improved diagnostic performance, yielding an AUC of 0.839 (0.749–0.928, $P < .001$). PLTP/UCr > 620.76 pg/mmol (78.8% sensitivity and 81.6% specificity)and F5/UCr > 20.19 pg/mmol (78.8% sensitivity and 67.3% specificity) can predict crescentic nephritis. Additionally, the AUC value of F5/UCr for predicting composite adverse renal outcomes was 1.01 ($P = .043$, Supplementary Table S3).

Neural network analysis

By neural network analysis, we observed that time interval from onset to diagnosis, hemoglobin, albumin, maximum creatinine level during hospitalization, urinary red blood cells (U-RBC), and urine F5/UCr ratio could predict the CrGN with an AUC of 0.984 on the training set (Supplementary Fig. S6) and 0.947 on the validation set. The accuracy of the confusion matrix reaches 95.5% on the training set and 72.7% on the validation set.

DISCUSSION

In this study, by DIA urine proteomic analysis, we identified the complement and coagulation cascades and neddylation played a crucial role in the CrGN with good prediction effect of F5 and PLTP, and provided potential non-invasive biomarkers in the diagnosis and management of CrGN, especially in cases where renal biopsy is not feasible or advisable.

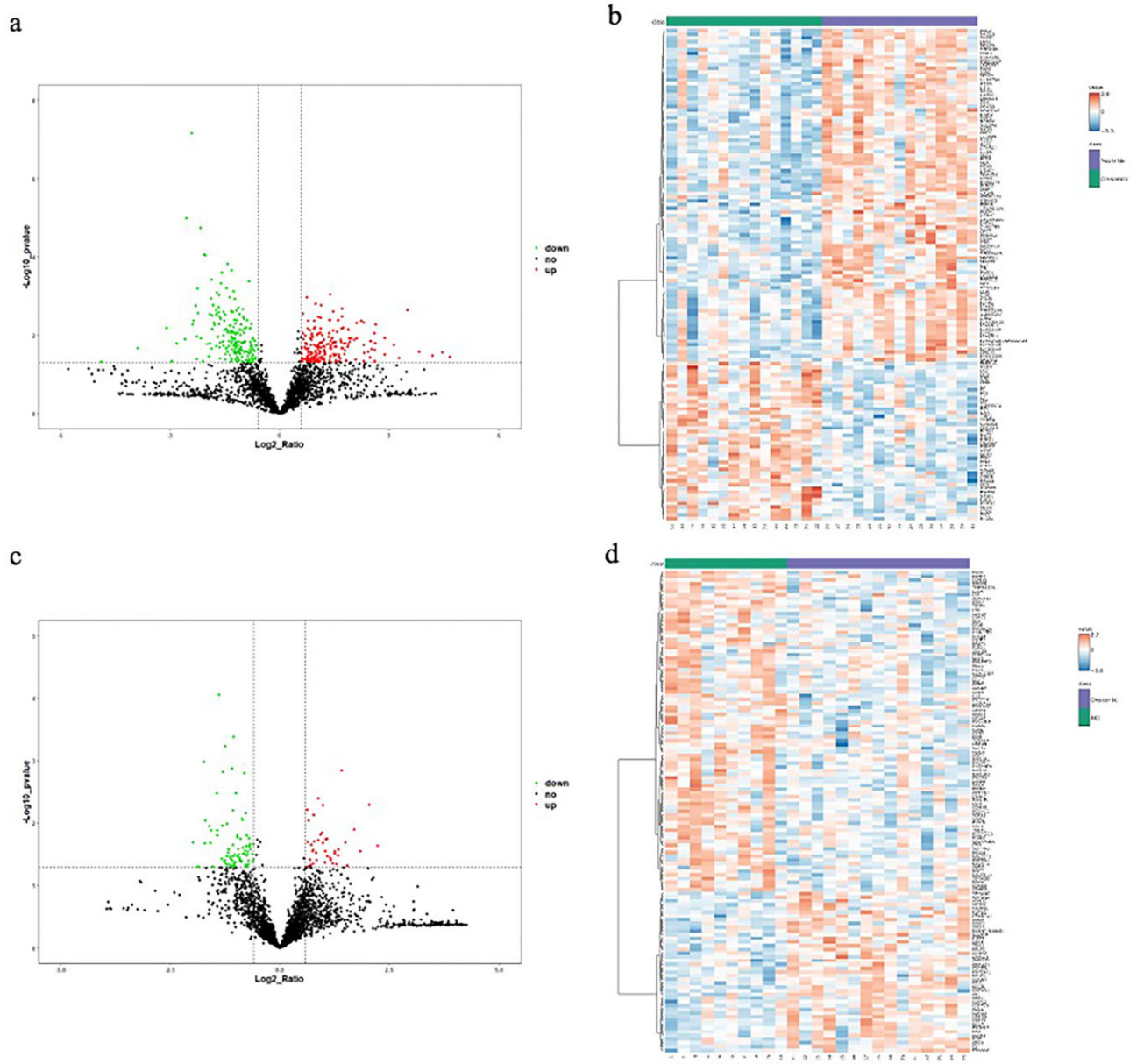


Figure 2: Proteomic differentiation. Volcano plot (**a**) and heatmap (**b**) comparing CrGN vs. non-crescentic nephritis groups. Volcano plot (**c**) and heatmap (**d**) comparing CrGN versus AKI groups. Red and blue points in volcano plots denote significantly upregulated or downregulated proteins ($|\log_2 FC| > 1$, FDR < 0.01). Heatmap rows represent proteins, columns represent individual samples; red and blue indicate higher and lower expression relative to the mean.

Clinically, CrGN is characterized by a nephritic syndrome rapidly progressing to ESRD and needs urgent therapy and the prognosis depending on the timeline of diagnosis [12]. The golden diagnosis standard is still the pathological diagnosis not available for emergency patients and aged patients. Traditionally, clinicians identify CrGN based on the characteristic features of rapid deterioration of renal function over a short period, accompanied by oliguria, gross hematuria, and anemia [12], more pronounced hypoalbuminemia, anemia. Recently, studies have attempted to explore more precise biomarkers for CrGN through minimally invasive approaches, such as FSP1 [4], urine soluble CD163 [13], and monocyte chemoattractant protein-1 [14]. However, urinary FSP1 is also in patients with glomerular injury caused by podocyte detachment without crescent formation, while urine soluble CD163 was not better than albumin as a marker for assessing glomerular barrier dysfunction [13]. Monocyte chemoattractant protein-1 and RANTES were associated with inflammation but with lower AUCs and limited mechanistic links to crescent formation [14]. Thus, it is still essential to identify new biomarkers for urgent clinical needs based on the general pathways and urgent clinical needs for applicable predictive models for CrGN and the prognosis.

This study identified that neutrophil degranulation, complement, coagulation cascade, and neddylation were the high-ranked pathways in the CrGN group. This finding is consistent with the mechanism studies of the CrGN published before that showed circulating cells, inflammatory mediators, and plasma proteins cross the fissures when severe inflammatory injury results in physical damage to the glomerular capillary wall, GN basement membrane, and renal capsule in the

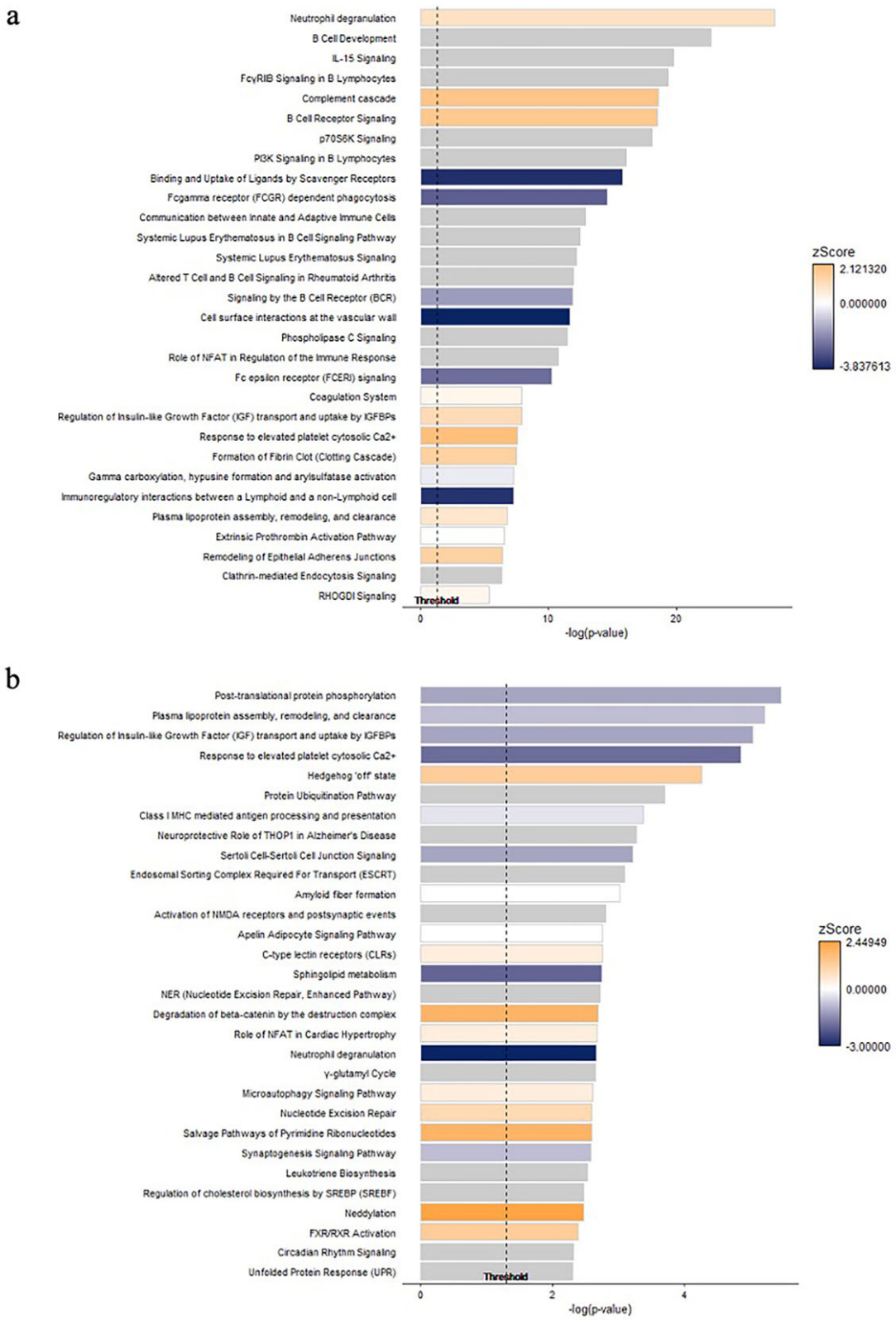


Figure 3: Pathway analysis. IPA of DEPs. (**a**) Top dysregulated pathways in CrGN versus nephritis controls: neutrophil degranulation (z-score = 2.1, P = 1.3E-18) and complement cascade (z-score = 2.0, P = 5.2E-12). (**b**) Top pathways in CrGN vs. AKI: neddylation (z-score = 2.3, P = 3.8E-4) and NF-κB signaling (z-score = 1.9, P = 7.1E-5). Orange and blue denote pathway activation or inhibition.

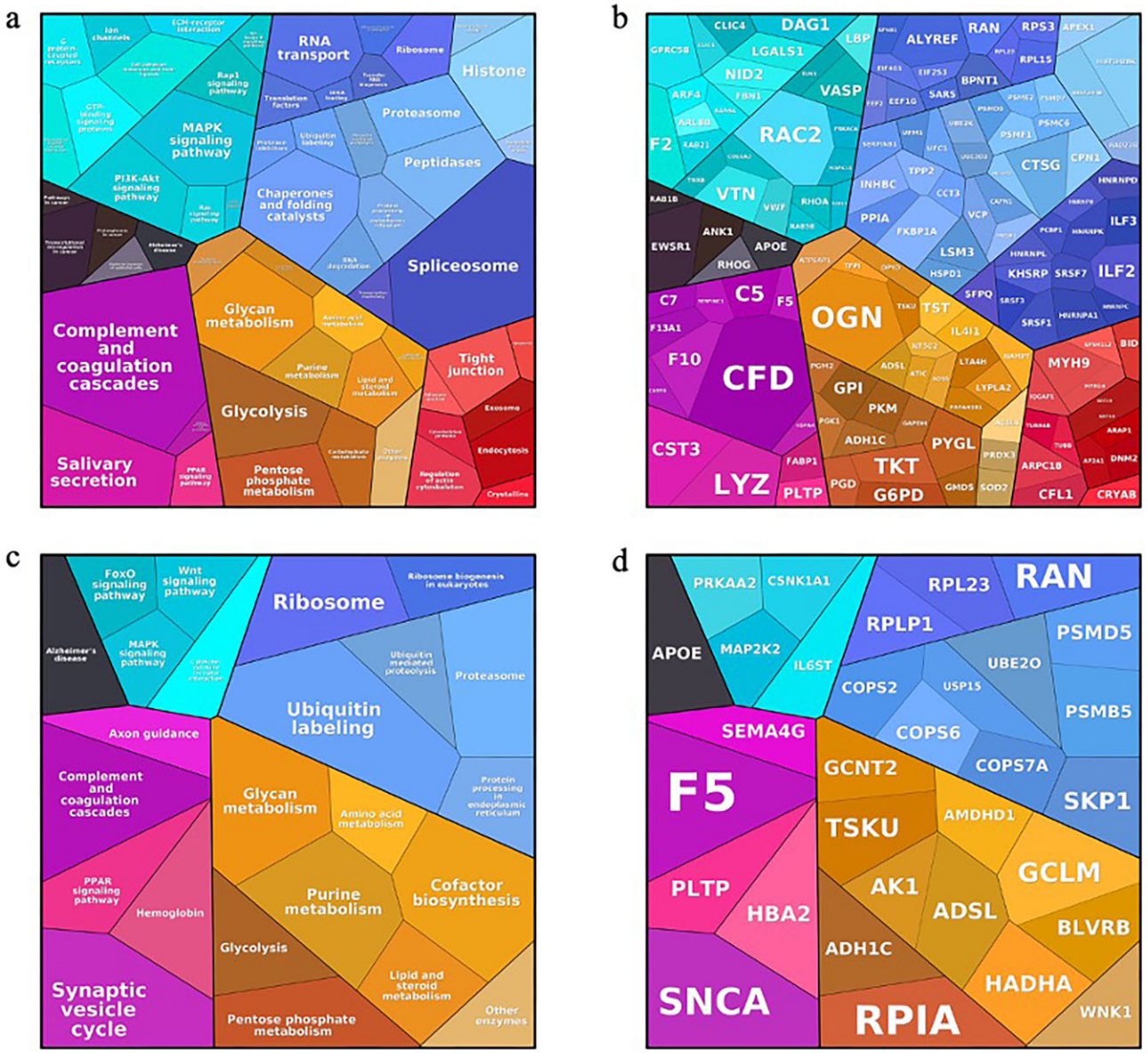
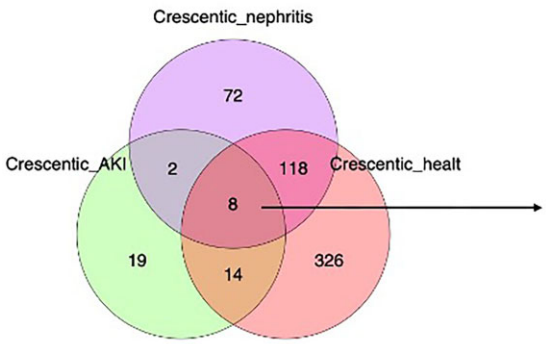


Figure 4: Proteomaps. The proteomaps of the differentiation between CrGN group, nephritis group (a, b) and AKI without the CrGN group (c, d), depicting the enriched pathways and corresponding proteins. Every tile (small polygon) represents one type of protein. Tiles are arranged and colored according to the hierarchical KEGG pathway maps.



F5	**Coagulation factor V**
PLTP	**Phospholipid transfer protein**
ADH1C	**Alcohol dehydrogenase 1C**
ADSL	Adenylosuccinate lyase
APOE	Apolipoprotein E
RAN	GTP-binding nuclear protein Ran
TSKU	Tsukushi
FN3KRP	Ketosamine-3-kinase

Figure 5: Overlapping DEPs. Venn diagram of DEPs (FDR < 0.01 and AUC > 0.8) common to CrGN versus nephritis (200 DEPs, purple), CrGN versus AKI (40 DEPs, green), and CrGN versus healthy groups (464 DEPs, pink). Eight overlapping DEPs were identified. Purple denotes DEPs between CrGN and nephritis group; pink, DEPs between CrGN and healthy controls; and green, DEPs between CrGN and AKI group.

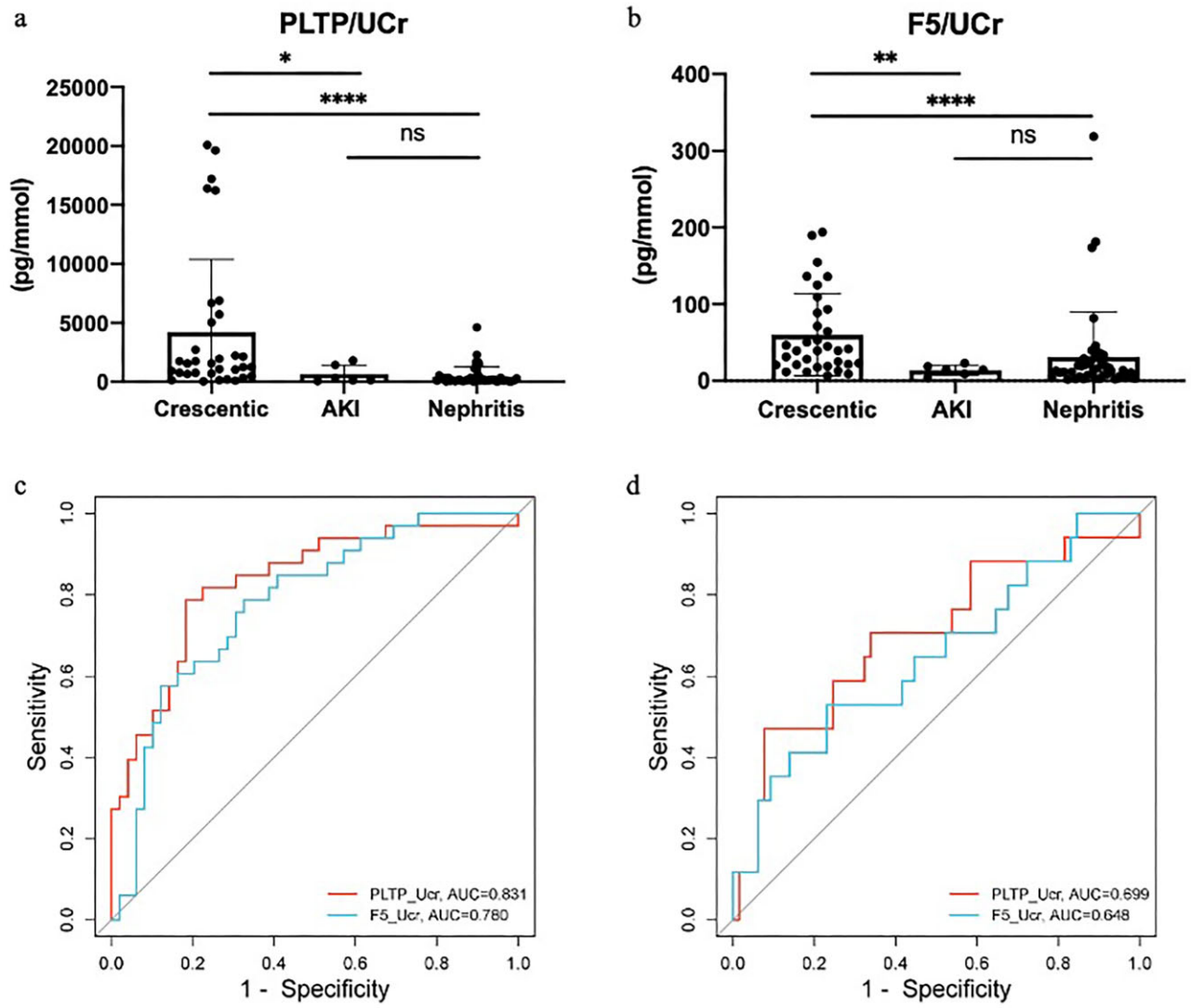


Figure 6: Biomarker validation. ELISA validation of urinary biomarkers normalized to creatinine (UCr). (**a**) PLTP/UCr and (**b**) F5/UCr levels in CrGN versus nephritis controls. ROC curves show diagnostic performance: PLTP/UCr AUC = 0.831 (95% CI: 0.738–0.924), F5/UCr AUC = 0.780 (0.679–0.881). Optimal cutoffs: PLTP/UCr > 620.76 pg/mmol (78.8% sens, 81.6% spec), F5/UCr > 20.19 pg/mmol (78.8% sens, 67.3% spec). Crescentic, crescentic nephritis group; Control, nephritis control group. ROC curves show diagnostic performance for predicting Crescentic Glomerulonephritis (**c**) PLTP/UCr AUC = 0.831 (95% CI: 0.738–0.924), F5/UCr AUC = 0.780 (0.679–0.881). Optimal cutoffs: PLTP/UCr >620.76 pg/mmol (78.8% sens, 81.6% spec), F5/UCr >20.19 pg/mmol (78.8% sens, 67.3% spec). Diagnostic performance for predicting composite adverse renal outcomes (**d**) PLTP/UCr AUC = 0.699, F5/UCr AUC = 0.648.

"general pathway" of crescent formation [15]. It also provides a mechanism base for the new biomarkers and the diagnosis and prognosis prediction.

We first discovered that urine F5/UCr and PLTP/UCr were promising indicators for predicting CrGN with high sensitivity and specificity. F5 is a crucial component of the coagulation cascade and is associated with GNs. In the rat model of mesangioproliferative GN, coagulation in the mesangial area promotes extracellular matrix accumulation through F5 expression [16]. Elevated urinary levels of F5 in CrGN patients might indicate the disruption of glomerular capillary integrity and active glomerular inflammation. PLTP transfers amphiphilic lipids between circulating lipoproteins, cells, and tissues associated with the immuno-response and inflammatory processes [17]. They were common pathogenesis of the causes of CrGN, such as AAV, anti-GBM disease, and lupus nephritis. In an RNA sequencing study of chronic kidney disease, PLTP was a novel biomarker of kidney fibrosis [18]. While F5 and PLTP emerged as consistent biomarkers of CrGN, their roles in disease pathogenesis remain speculative. The associations observed in this study should be considered hypothesis-generating, and functional studies are needed to evaluate their mechanistic relevance.

The machine learning model demonstrates significant discriminative ability in predicting CrGN in the training (AUC = 0.984) and validation (AUC = 0.947) cohorts. It suggests that the model can effectively differentiate between cases of CrGN and other causes of AKI, which provides evidence for urgent therapy when pathologic diagnosis is unavailable. The model's strength lies in the cooperation of clinically relevant features, including time intervals from onset to diagnosis, hemoglobin, albumin, maximum creatinine level during hospitalization, U-RBC, and urine F5/UCr ratio. In the IgAN, urinary protein, U-RBC, eGFR <60 ml/min/1.73 m^2, and serum IgA/C3 ratio have been reported as independent risk factors for crescent

formation [19] with accuracies in training (95.5%) and validation (72.7%), suggests some degree of overfitting. Further validation of external datasets and prospective studies would be necessary to ensure robustness and generalizability.

These urinary biomarkers hold promise for a strategy of non-invasive diagnosis and monitoring of CrGN, particularly in cases where renal biopsy is contraindicated or unavailable. Furthermore, these biomarkers may provide insights into the pathogenic mechanisms driving the rapid progression of glomerular nephritis, then develop potential targeted therapeutic strategies for the rare and severe renal disease in the clinical setting. To expand the sample size and improve the representativeness of the validation cohort, patients were consecutively enrolled over a 5-year period in a retrospective-prospective manner. Therefore, strict age matching between the training and validation cohorts was not implemented. The observed differences in age distribution between cohorts likely reflect underlying heterogeneity in clinical presentation and enhance the robustness and external validity of the identified urinary proteomic biomarkers. Future studies with larger cohorts are warranted to dissect the differential proteomic signatures between inflammation-associated injury and non-inflammatory AKI, which may further refine biomarker specificity. Another limitation of our study is the predominance of Type III CrGN, primarily AAV, in both the training and validation cohorts. This imbalance may limit the generalizability of our findings to other CrGN subtypes, such as Type I (anti-GBM disease) and Type II (immune-complex mediated CrGN). Future studies should aim to include a broader distribution of CrGN types to validate the biomarker performance across the full disease spectrum.

CONCLUSION

By the non-invasive urine proteomics, the new biomarkers F5/UCr and PLTP/UCr shed light on identifying the CrGN patient. Complement, coagulation cascades, and neddylation might be critical in activating the crescentic nephritis. A newly constructed neural network model to predict CrGN could promote clinical decision-making.

SUPPLEMENTARY DATA

Supplementary data are available at *Clinical Kidney Journal* online.

FUNDING

This work was partially supported by grants from the National Key R&D Program of China (2022ZD0116003 to C.L.); National Natural Scientific Foundation of China (82170709 to C.L., Youth Program 82200814 to Z.S); CAMS Innovation Fund for Medical Sciences (CIFMS 2021-I2M-1-003 to C.L.); National High-Level Hospital Clinical Research Funding (2022-PUMCH-A-174 to Z.S.); the Capital Exemplary Research Wards Project (BCRW202001 to C.L.); Beijing Natural Science Foundation (7252112 to C.L.); and CAMS Innovation Fund for Medical Sciences (CIFMS 2024-I2M-C&T-C-003, CIFMS 2021-I2M-1-003 to C.L.). The funders had no role in study design, data collection and analysis, the decision to publish, or the preparation of the manuscript.

AUTHORS' CONTRIBUTIONS

S.Z. and X.C. were responsible for the conception and design of the study. X.C. drafted the manuscript. Y.H., Y.W., and P.X. were responsible for data acquisition and analysis. J.X. and B.Q. provided the technical support for the urine proteomics analysis. L.C. read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The mass spectrometry proteomics data have been deposited in the National Genomics Data Center (NGDC, https://ngdc.cncb.ac.cn/) under accession number OMIX011110. Associated metadata, including patient groupings, experimental conditions, and data processing parameters, are also available in the repository.

CONFLICT OF INTEREST STATEMENT

None declared.

STATEMENT OF ETHICS

The Ethics Committee of PUMCH (No. K2185) reviewed and approved the studies involving human participants.

CONSENT STATEMENT

Written informed consent was obtained from all participants. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

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Received: 21.3.2025; accepted: 9.9.2025