Urinary Protein and Peptide Markers in Chronic Kidney Disease

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Abstract: Chronic kidney disease (CKD) is a non-specific type of kidney disease that causes a gradual decline in kidney function (from months to years). CKD is a significant risk factor for death, cardiovascular disease, and end-stage renal disease. CKDs of different origins may have the same clinical and laboratory manifestations but different progression rates, which requires early diagnosis to determine. This review focuses on protein/peptide biomarkers of the leading causes of CKD: diabetic nephropathy, IgA nephropathy, lupus nephritis, focal segmental glomerulosclerosis, and membranous nephropathy. Mass spectrometry (MS) approaches provided the most information about urinary peptide and protein contents in different nephropathies. New analytical approaches allow urinary proteomic–peptide profiles to be used as early non-invasive diagnostic tools for specific morphological forms of kidney disease and may become a safe alternative to renal biopsy. MS studies of the key pathogenetic mechanisms of renal disease progression may also contribute to developing new approaches for targeted therapy.

Keywords: biomarkers; urine; proteomics; chronic kidney disease

1. Introduction

According to The Kidney Disease: Improving Global Outcomes (KDIGO) criteria, chronic kidney disease (CKD) is defined as an abnormality in kidney structure or function present for more than 3 months, with health implications [1,2]. CKD is an independent risk factor for death, cardiovascular disease, end-stage renal disease, and acute kidney injury [3–7] and has a global prevalence of 11–13% [8]. CKD is a socially significant problem due to the high risk of early disability from the disease and the need for high-cost treatments in the case of end-stage renal failure, such as hemodialysis, peritoneal dialysis, and kidney transplants [9,10]. The three most common causes of CKD are diabetes mellitus, hypertension, and glomerulonephritis, especially with nephrotic syndrome [11]. Kidney diseases can have similar clinical symptoms and may range from mild and benign to progressive with rapid end-stage renal disease development. The severity of the clinical manifestations, however, does not always correspond to the severity of renal damage, which can be determined by renal biopsy [12]. The majority of patients undergo a single kidney biopsy to determine the morphological form of kidney disease. In sporadic cases, the biopsy is repeated to assess the effectiveness of therapy and prognosis. However, the assessment of the regression of nephropathic activity is crucial for the dynamic assessment of treatment, including the treatment’s effectiveness, optimization, and prognosis.
Urine proteomic analysis is a much safer option compared to a biopsy and has good potential for developing non-invasive diagnostic methods. Urine analysis has several advantages compared to blood proteomic analysis [13]. Firstly, the urine proteome is not very complicated and mainly contains proteins and peptides of renal origin (up to 70%). On the contrary, kidney damage markers comprise only a small fraction of the highly diverse plasma/serum proteome, making their analysis in the latter challenging. Secondly, it is much easier to normalize the concentration of a protein biomarker in the urine than in the blood—for example, based on the concentration of creatinine [14]. Thirdly, urine collection is simple and non-invasive. Finally, urine samples are stable at a temperature of $-20^\circ C$ and are suitable for proteomic analysis even after years of storage [15]. The aforementioned advantages of urine make it a popular subject for the search for protein markers for various pathologies [16]. These pathologies include renal and genitourinary pathologies and pathologies associated with proteinuria, such as kidney diseases [17–19]; bladder, prostate, and ovarian cancers [20–23]; diabetic nephropathy [24]; and pre-eclampsia [25–27]. Urinary protein markers have also been described for colon and lung cancers [28,29], cholangiocarcinoma [30], cardiovascular diseases [31], autoimmune diseases [32], and infectious diseases [33]. Nevertheless, the urine proteome should be most informative for renal pathologies and may present a fingerprint of different kidney diseases [34–39] (Table 1).

However, despite a large number of studies, there are still no reliable kidney-disease-specific biomarkers that can be accurately reproduced in different studies. The various factors affecting proteome composition include the collection conditions and regime (morning, daily, variability over several days, etc.), physical activity, nutrition, the anatomical features of the urinary tract (the absence of one kidney, etc.), sex, and age [40–43]. All of these factors should be taken into account when comparing the results of different studies. In general, combining the markers of specific nephropathies outlined in various studies could facilitate better progress in the creation of highly specific differentiating panels for possible clinical use after multi-stage prospective validation [44].

Mass spectrometry (MS)-based approaches, which feature a high multiplexing capacity, are the most unbiased and sensitive instruments and have already provided most of the currently known information about urine peptide and protein contents in different nephropathies, as well as potential biomarker panels for various diseases [37–39]. A number of MS methods have been successfully applied (Table 1). The most commonly used approaches include matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), capillary electrophoresis (CE), and liquid chromatography (LC) MS. The most advanced approaches with isobaric or tandem mass tags for relative and absolute quantitation (iTRAQ and TMT) facilitate the identification of markers among commonly present proteins and peptides when their amounts vary significantly. In general, the listed untargeted MS approaches are the most appropriate for the primary search of potential biomarkers, whereas targeted MS and immunoassays can be used for further validation.

This review summarizes data from numerous studies of the urine proteome in nephropathies associated with CKD, with a focus on recent studies from 2015 to 2021. The electronic databases MEDLINE, PubMed, and Cochrane were searched using keywords such as “proteomics”, “peptidomics”, “biomarkers”, “chronic kidney disease”, “urine”, “membranous nephropathy” “IgA nephropathy”, “focal segmental glomerulosclerosis” “minimal-change disease”, “diabetic nephropathy”, and “lupus nephritis”. The reference lists of articles were also investigated to explore related literature. The bibliographic information of 1030 retrieved articles was analyzed, and papers with irrelevant or unreliable information, those unavailable in full text, and those not in English were deleted. After deleting all duplicate references, 69 articles remained. A flow chart is outlined in Figure 1.
Figure 1. Study flow chart.

Table 1. Urine proteome studies in different types of nephropathies.

| Nephropathy Types            | Method     | Number of Patients | The Main Biomarkers                                                                 | Functions of Proteins/Main Processes                  | References                        |
|------------------------------|------------|--------------------|------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------|
| Chronic kidney disease       | CE-MS      | 4766               | CKD 273 classifier<br>↓ fragments of different collagens, ↑ A1AT, serum albumin, hemoglobin α chain, fibrinogen α chain, uromodulin, Na⁺/K⁺-ATPase γ chain, and membrane-associated progesterone receptor component 1 | CKD progression and fibrosis accumulation             | Good et al., 2010 [45]            |
|                              | CE-ESI-TOF MS | 1028               | CKD 273 classifier validation                                                      |                                                      | Puntillo et al., 2017 [46]        |
|                              | CE-ESI-TOF MS | 1990               | CKD 273 classifier validation                                                      |                                                      | Schansstra et al., 2015 [18]      |
|                              | CE-MS      | 435                | FPP_29BH classifier<br>↑ cathepsin D, MMP-2, collagenase 3, MMP-14, α-2-HS-glycoprotein, fetuin-A, and 19 different collagen peptide fragments | Fibrosis accumulation                                 | Catanese et al., 2021 [47]        |
### Table 1. Cont.

| Nephropathy Types | Method | Number of Patients | The Main Biomarkers | Functions of Proteins/Main Processes | References |
|-------------------|--------|--------------------|---------------------|--------------------------------------|------------|
| **FSGS/MCD**      | 2D-DIGE-MS | 49                 | A1AT, transferrin histatin-3, 395 ribosomal protein L17 ↓ (FSGS vs. MCD), calretinin ↑ (FSGS vs. MCD) | Modulating immunity, inflammation and apoptosis; abnormal permeability of GBM, cell proliferation and differentiation | Perez et al., 2017 [48] |
|                   | 2D-LC-MS/MS | 30 FSGS, 30- MCD   | Ubiquitin-60S ribosomal protein L40 (UBA52) (FSGS vs. MCD) | Protein degradation, stress response, and overexpression of UBA52 ameliorated the cell-cycle arrest | Wang et al., 2017 [49] |
|                   | LC-MS/MS   | 4 MCD, 4 FSGS      | CD14, C9, and A1AT, cadherin-like 26, RNase A Family 1, DIS3-like exonuclease 1 | Complement activation, inflammation, apoptosis, cells adhesion, and cell death | Choi et al., 2017 [50] |
|                   | nano-LC-MS/MS | 10 FSGS             | ↑ Apolipoprotein 1, matrix-remodeling protein 8 (MXRA8) | Lipid oxidation and matrix accumulation | Kalantari et al., 2014 [51] |
|                   | nanoLC-MS/MS | 11 FSGS             | ↑ DPEP1, CD59, CD44, insulin-like growth factor-binding protein 7, and roundabout homolog 4 | | Nafar et al., 2014 [52] |
|                   | MALDI-MS imaging | 6 FSGS             | A1AT | Marker of podocyte stress, excessive loss, and hypertrophy of podocytes and glomerulosclerosis | Smith et al., 2016 [53] |
|                   | CE-MS      | 110 FSGS, 35 MCD   | ↑ Collagens, A1AT, ↓ clusterin, uromodulin polymeric immunoglobulin receptor, Golgi-associatedolfactory signaling regulator, ↑ collagens, uromodulin, keratin apolipoprotein C-IV, ↓ β-2-microglobulin, clusterin, complement C3 | | Siwy et al., 2017 [54] |
| Membranous nephropathy | CE-MS | MCD 14, DN 11 | retinol-binding protein 4 and SH3 domain-binding glutamic acid-rich-like protein 3 | Proteins could distinguish between MCNS and DN | Araumi et al., 2021 [55] |
|                   | LC-MS/MS   | 4                  | SERPINA7 and CD44 | Cell–cell interactions, cell adhesion, and migration | Choi et al., 2017 [50] |
|                   | iTRAQ and LC-MS/MS | 5              | Lysosome membrane protein-2 | Immune inflammation | Rood et al., 2015 [56] |
|                   | TMT1 and TMT2+ nanoLC-MS/MS | 63         | A1ATafamin | Contribute to accumulation of mesangial matrix lipid metabolism | Pang et al., 2018 [57] |
|                   | MALDI-TOF MS | 13                | ↑ UMOD, ↑ A1AT | Tubular dysfunction inflammation and apoptosis; matrix accumulation | Navarro-Muñoz et al., 2012 [58] |
| Nephropathy Types | Method          | Number of Patients | The Main Biomarkers                                                                 | Functions of Proteins/Main Processes                                                                 | References                |
|------------------|-----------------|--------------------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|---------------------------|
| CE-MS            | 77              | ↑ A1AT, uromodulin, α-1B-glycoprotein, plasminogen, keratin, apolipoprotein C-IV ↓ Fibrinogen α-chain, zinc finger protein ZFPM2, E1A-binding protein Microtubule-associated protein tauAP-3 complex subunitdelta-1 | Could distinguish between MN and DN                                                                   | Siwy et al., 2017 [54]    |
| CE-MS            | 23              | The combination of urinary afamin and complement C3 urine/plasma ratio                  | Complement activation; glomerular filtration barrier damage                                         | Araumi et al., 2021 [55]  |
| iTRAQ-MS         | 4               | Complement C9, Ig kappa chain C region, cytoskeletal keratins type I (10), and type 2 (1, 5) | Inflammation; urine proteins originated from serum leakage                                         | Ning et al., 2017 [59]    |
| 2D-LC-MS/iTRAQ   | 12              | ICAM 1, metalloproteinase inhibitor 1, antithrombin III, and adiponectin                 | Inflammation; urine proteins originated from serum leakage                                         | Guo et al., 2018 [60]     |
| MALDI-TOF/TOF    | 20              | ↓ UMOD ↑ A1AT                                                  | Accumulation of mesangial matrix                                                                   | Prikryl et al., 2017 [61] |
| CE-MS            | 209             | ↓ Collagen I ↑ A1AT                                           | Matrix accumulation and glomerulosclerosis                                                          | Rudnicki et al., 2020 [62]|
| IEF/LC-MS/MS     | 30              | ↑ α-2-macroglobulin, ceruloplasmin, complement C3, complement C4a, haptoglobin, prothrombin, and antithrombin-III | Coagulation, complement activation, and cell interaction in inflammation                          | Mucha et al., 2014 [63]   |
| 2D-DIGE-MALDI-TOF/TOF | 43   | Albumin fragments, A1AT, and α-1- β-glycoprotein ↓ laminin G-like 3 (LG3) fragment of endorepellin | Matrix accumulation of endothelial cells; extensive fibrosis                                       | Surin et al., 2013 [64]   |
| nanoLC-MS/MS     | 13              | CD44, glycoprotein 2, vonhiperderal growth factor, CMRF35-like molecule 5, proteodaherin, uroeglobin, dipeptidyl peptidase IV, NHl repeat-containing protein 3, SLAM family member 5 (CD84) | Activation of apoptosis, immune inflammation, coagulation, and complement                          | Samavat et al., 2015 [36]|
| LC-MS/MS         | 24              | ↓ Aminopeptidase N and vonasiron precursor levels were higher on average in the urinary exosome samples ↑ A1AT and ceruloplasmin | IgAN markers vs. thin basement membrane nephropathy                                               | Moon et al., 2011 [65]    |
| CE-MS            | 179             | ↑ Small proline-rich protein, leucine-rich repeat-containing protein, A1AT, sodium/potassium-transporting ATPase subunit gamma | Effect on inflammation of proximal renal tubule function                                            | Siwy et al., 2017 [54]    |
| SELDI-TOF        | 49 inactivet 26 active | Protein ions with m/z of 3340 and 3980 | Distinguished active from inactive LN                                                             | Mosley et al., 2006 [66]  |
| SELDI-TOF        | 19 active       | Hepcidin, fragments of A1AT, and albumin                                                                       | Infiltration interstitial leukocytes, cytokines production, and matrix accumulation               | Zhang et al., 2008 [67]   |
| CE-MS            | 92              | Collagens, uromodulin, protein S100-A9, clusterin, β-2-microglobulin, and α-2- HS-glycoprotein | Matrix accumulation                                                                                 | Siwy et al., 2017 [54]    |
| 2D-DIGE-MALDI-TOF/MS/MS | 88      | Haptoglobin, α-1 anti-chymotrypsin, and retinol-binding protein Effect on inflammation loss of proximal renal tubule function |                                           | Aggarwal et al., 2017 [68]|
| iTRAQ-MS         | 61              | α1-antichymotrypsin (SERPINA3) Marker of LN activity                                                           |                                                                                                   | Turnier et al., 2019 [69] |

**Table 1. Cont.**
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| Nephropathy Types | Method | Number of Patients | The Main Biomarkers | Functions of Proteins/Main Processes | References |
|-------------------|--------|--------------------|---------------------|--------------------------------------|------------|
| CE-MS             | 93     | CKD273 validation  | It could not identify urinary biomarkers and predict active LN | Tailliari et al., 2021 [70] |
| 2D-DIGE-LC-MS/MS  | 33     | ↑ α1B-Glycoprotein zinc-α2-glycoprotein, α2-HS-glycoprotein vitamin D-binding protein (VDBP), calgranulin B, A1AT, hemopexin ↓ Transthyretin, apolipoprotein A1, AMBP, and plasma retinol-binding protein | Hyperglycosylated state and matrix accumulation | Rao et al., 2007 [71] |
| CE-MS             | 305    | ↓ Collagen type I and uromodulin fragments ↑ albumin | Increased synthesis of protease inhibitors diminishes excretion of collagen fragments | Rossing et al., 2008 [72] |
| CE-MS             | 126    | CKD 273 classifier | Accumulation of proteins in the extracellular matrix chronic renal damage | Good et al., 2010 [45] |
| CE-MS             | 576    | ↑ Clusterin, apoliprotein ↓ hemoglobin, uromodulin, small proline-rich protein 3, leucine-rich repeat-containing protein 25 | Different stage of DN | Siwy et al., 2017 [54] |
| iTraq             | 50     | 408 N-linked glycoproteins, A1AT, and ceruloplasmin | Different stage of DN | Jin et al., 2020 [73] |
| 2D-DIGE-MALDI Q-TOF | 268   | Transthyretin/prealbumin and Ig kappa C chain region + cystatin C, and ubiquitin + α1-acid glycoprotein 1, apolipoprotein A1, α1 microglobulin/bikunin precursor, pigment epithelium-derived factor, zinc α-2-glycoprotein | 0–5 years of T2DM duration 5–10 years more than 10 years | Patel and Kalia, 2019 [74] |
| iTraq             | 65     | ↑ Haptoglobin and α-1-microglobulin/bikunin precursor | Proximal tubular dysfunction | Liao et al., 2018 [75] |
| C18 plate-MALDI-TOF | 174   | ↑ β2-microglobulin and clara-cell protein | Proximal tubular dysfunction | Chen et al., 2018 [76] |

CE—capillary electrophoresis; DIGE—differential in-gel electrophoresis; ESI—electrospray ionization; IEF—isoelectric focusing; iTraq—isobaric tags for relative and absolute quantitation; MALDI—matrix-assisted laser desorption/ionization; SELDI—surface-enhanced matrix assisted laser desorption/ionization; TOF—time-of-flight; TMT—tandem mass tags.

2. Chronic Kidney Disease (CKD)

Several urinary proteome studies have considered the CKD group of pathologies without subdivisions. Harald Mischak’s group is the leader in MS studies on the urinary peptidome and proteome. This group described 1580 native urinary peptides, showing that 73% were unique for urine and proving the clinical value of native urinary peptide markers for diagnosing several diseases, including those associated with kidney damage [14,77,78].

Rossing K. et al. developed the first panel consisting of 65 urinary proteins, including collagen fragments, serum albumin, α1-antitrypsin (A1AT), and uromodulin, which differentiated diabetic nephropathy in 97% of cases, showing high sensitivity and specificity among 148 type 2 DM and DN patients [72]. The panel was further successfully validated by Alkhalaif A. et al. [79].

Good D.M. et al. analyzed urine samples from 476 patients with CKD (mostly diabetic nephropathy) and 379 controls and developed a classifier based on 273 urinary peptides (CKD273) in the form of a composite CKD biomarker [45]. The panel contained fragments of collagen type I and III α-chains (181 peptides), reflecting the extracellular matrix turnover and reduced protease activity in situ. CKD patients also demonstrated increased urinary excretion of plasma proteins and their fragments (e.g., A1AT, serum albumin, α-hemoglobin chain, and α-fibrinogen chain), kidney-specific proteins (uromodulin, gamma-chain Na+/K+-ATPase, and membrane-associated progesterone receptor component 1), and proteins excreted by the tubules, which may reflect chronic damage to the glomerular filtration barrier, increased glomerulosclerosis, and interstitial fibrosis. In a
blinded study, the CKD273 classifier made it possible to differentiate patients with CKD of various etiologies with 85.5% sensitivity and 100% specificity and predict the mortality in type 2 DM with microalbuminuria [45,80,81]. These results were further validated by Schanstra J.P. et al. [18] and Pontillo C. et al. [46], who confirmed this classifier’s value as a predictor of renal function deterioration and demonstrated a decrease in the glomerular filtration rate of <60 mL/min over 5 years of monitoring. Zürbig P. et al. showed that the CKD273 classifier could predict the development of diabetic nephropathy 1.5 years before the onset of microalbuminuria. Argiles A. et al. used the CKD273 classifier on 53 patients with CKD and differentiated patients according to their degrees of impairment of renal function and the risk of end-stage CKD or death [17].

Catanese L. et al. developed the FPP_29BH classifier, which contains 29 specific fibrosis biomarkers for patients with various immune and non-immune kidney diseases. The patients with renal fibrosis showed an increase in urinary proteases (cathepsin D, matrix metalloproteinase 2, collagenase 3, and matrix metalloproteinase-14), α-2-HS-glycoprotein, or fetuin-A, as well as 19 different collagen peptide fragments of eight different collagen chains with differential intensities between patients with high and low degrees of fibrosis [47].

Since CKD is an umbrella term for several conditions that affect the kidneys, many of the aforementioned markers are not disease-specific. A study of 1180 urine samples by Siwy J. et al. showed that many markers remain the same in different nephropathies and reflect common pathological processes [54]. However, this large-scale study identified a number of specific markers. Three fragments of clusterin were shown to be increased in diabetic nephropathy, β-2-microglobulin was decreased in minimal-change disease, and a S100-A9 protein fragment distinguished lupus nephritis [54]. Other specific proteomic and peptidomic changes in various CKD types, including minimal-change disease (MCD), focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MN), IgA nephropathy (IgAN), diabetic nephropathies (DN), and lupus nephritis (LN), are reviewed below.

3. Minimal Change Disease and Focal Segmental Glomerulosclerosis

Minimal-change disease (MCD) and primary focal glomerulosclerosis (FSGS) are diseases with primary podocyte injury (primary podocytopathies), which is manifested as high proteinuria and nephrotic syndrome [82,83]. However, morphological studies of a kidney biopsy in the early stages of FSGS can miss segmental sclerosis in individual glomeruli and may misclassify the disease as MCD [84]. Primary FSGS pathogenesis is associated with circulating permeability factors (such as soluble urokinase-type plasminogen activator receptor (SuPAR), cardiophthrin-like protein-1, and anti-CD40 antibody CD-80 expression), which leads to the development of nephrotic syndrome [85–91]. In general, compared to FSGS, MCD has a more favorable prognosis regarding the progression of renal dysfunction; FSGS is more likely to develop therapy resistance and result in rapid renal dysfunction and is also more likely to need an aggressive and persistent therapeutic strategy [83,92,93]. In addition, the presence of secondary FSGS complicates diagnosis and disease treatment. Due to its non-immune nature, this form of the disease does not require immunosuppressive therapy [1].

Several studies have aimed at identifying proteomic differences between these two nephropathies. In particular, it was shown that the calretinin and UBA52 levels were higher in FSGS [48,49], while the 395 ribosomal protein L17 was higher in MCD [48] (Table 2). Significantly higher levels of cathepsin B, cathepsin C, and annexin A3 were shown in cases of the collapsing variant of FSGS (characterized by glomerular collapse and a rapid loss of renal function) than in MCD, MN, and other FSGS variants [94]. Several potential markers specific only for FSGS include increased levels of cadherin-like 26, RNase A family 1, DIS3-like exonuclease 1 [50], matrix-remodeling protein 8 [51], CD59, insulin-like growth factor-binding protein 7, and roundabout homolog 4 [52], as well as a decrease in the polymeric immunoglobulin receptor and Golgi-associated olfactory signaling regulator [54] or the complete absence of dipeptidase 1 (DPEP1) [52]. Increased CD14 levels were found
to be specific only for MCD [50] and were not identified in any other nephropathy (Table 2). At the same time, increases in transferrin and histatin-3 may distinguish both FSGS and MCD [48] from other types of kidney disease.

Among the revealed potential markers, the overrepresentation of ribonuclease 2 and underrepresentation of haptoglobin may suggest the worst FSGS prognosis, whereas apolipoprotein A1 and matrix-remodeling protein 8 (MXRA8) showed significant changes between steroid-sensitive and steroid-resistant forms of FSGS [51].

In general, the presence of most of the aforementioned proteins in the urine and increases in their levels may reflect massive cell death and the release of intracellular contents during the podocytes’ separation from the glomerular membrane. These results may also suggest special roles for immunity, inflammation, and apoptosis in the development of FSGS. Cell proliferation, differentiation, and death may be involved in MCD development [95]. Dynamic studies performed using a focal segmental glomerulosclerosis rat model (adriamycin (ADR)-induced nephropathy) revealed a gradual increase in afamin and ceruloplasmin, as well as a gradual decrease in cadherin-2 and aggregan core protein in FSGS, and suggested that decreased levels of fetuin-B, α-1-microglobulin, and α-2-HS-glycoprotein may be promising markers for the early detection of FSGS [96]. Other promising markers include CD44, MXRA8, cathepsins, and apolipoprotein A1. CD44 reflects oxidative stress, is associated with hyperlipidemia, and represents one of the pathogenetic factors in the development of FSGS.

4. Membranous Nephropathy

Membranous nephropathy (MN) is a leading cause of nephrotic syndrome (NS) in adults. This disease has an autoimmune nature, which was confirmed by the presence of autoantibodies to podocyte antigens, including antibodies to phospholipase A2 receptors (aPLA2R) and thrombospondin 1 domain-containing 7A (THSD7A) [97,98]. The secondary causes of MN include drug use, infections, autoimmune diseases, and cancer [99]. The primary mechanism of MN is podocyte autoimmune damage by phospholipase A2 receptor antibodies, leading to massive proteinuria. The diagnosis and treatment of this disease are currently based on the determination of the aPLA2R antibody titer. The search for additional markers seems promising in the aPLA2R-negative type of idiopathic MN.

MN patient studies provide comparative cross-sectional analyses of the proteome in MN compared to that in other nephrotic types of nephritis and healthy controls. The panel of specific urinary protein markers distinguishing MN from other nephropathies includes decreased levels of zinc finger protein ZFPM2, E1A-binding protein, and microtubule-associated protein tauAP-3 complex subunit delta-1 [54], as well as increased levels of thryroxine-binding globulin (SERPINA7) [50], lysosome membrane protein-2 (LIMP-2) [56], plasminogen [54], LDB3, PDLI5 [100], and afamin [55,57]. A comparison of samples from patients with APLA2R-positive MN and APLA2R-negative MN, as well as healthy individuals, revealed significantly higher levels of A1AT and afamine in the positive-MN group [101]. A combination of urinary retinol-binding protein 4 and SH3 domain-binding glutamic acid-rich-like protein 3 can differentiate MCD from DN. Similarly, a combination of urinary afamin and complement C3 urine/plasma ratio can differentiate MN from DN [55].

In general, markers found in MN play a role in the classical pathway of complement activation and immune responses, cell adhesion, receptor-mediated endocytosis, platelet degranulation, and the coagulation cascade [57]. LIMP-2 plays a pivotal role in inflammatory immune-response regulation in the kidney tissue [56] and reflects tissue infiltration by immune cells. LIMP-2 may also help to determine disease activity. The LDB3 and PDLI5 proteins play a role in the modification of the podocyte cytoskeleton, which can lead to proteinuria. Afamin, whose elevation is associated with idiopathic MN, is the
most promising specific MN marker, as its significance was confirmed in several studies (Table 2).

5. IgA Nephropathy

IgA nephropathy (IgAN) is the most common form of chronic glomerular disease in adults. In Europe, the frequency of IgAN ranges from 19 to 51% of the renal biopsies performed for glomerular diseases [102–104]. Patients with IgAN often have increased levels of IgA1 with galactose-deficient O-glycans in the hinge region. The blood levels of an aberrantly glycosylated IgA1 are higher in IgAN than in healthy controls or patients with other kidney diseases. The production of galactose-deficient IgA1 antibodies, immune-complex formation, and the accumulation of these complexes in the mesangium were shown to initiate renal injury [105]. Moreover, the activation of alternative complement pathways potentiated tissue injury [106]. Transferrin receptor (CD71) on human mesangial cells can bind immune complexes containing galactose-deficient IgA [107].

About 40 urinary protein markers differentiating IgAN have been described, >20 of which are specific only for IgAN (Table 2). The levels of complement C9, Ig kappa chain C region, and three cytoskeleton keratins (type I(10) and type II (1 and 5)) changed synchronously in the glomeruli (biopsy sample) of IgAN patients compared to the intact renal-tissue areas of patients with tumors [39]. Altered levels of 30 urine proteins and four potential markers (intercellular adhesion molecule 1 (ICAM1), metalloproteinase inhibitor 1, antithrombin III, and adiponectin) were revealed in IgAN with low proteinuria (<1 g/L) and stable renal function (glomerular filtration rate: 57.3 (23–106) mL/min). A larger multicenter study suggested that a decreased number of collagen fragments in the urine (specifically type I collagen) might be most informative in progressive IgAN, due to decreased collagen degradation and collagenase inhibition in kidney fibrosis [62].

Other potential IgAN-specific markers include increased levels of adiponectin [60], α2-macroglobulin, complement C4a, prothrombin [63], antithrombin III [60,63], α1B-glycoprotein [64], glycoprotein 2, epidermal growth factor, CMRF35-like molecule, protocadherin, utreoglobin, dipeptidyl peptidase IV, NHL repeat-containing protein 3, and CD84 [36] and decreased levels of transthyretin [71,74] and differently changing levels of the α1-microglobulin/bicunin precursor (AMBP) [71,74,75].

Significant increases in the urinary levels of α1B-glycoprotein (7-fold), zinc-containing α2-glycoprotein (5.9-fold), α2-HS-glycoprotein (4.7-fold), vitamin D-binding protein (4.8-fold), calgranulin B (3.9-fold), A1AT (2.9-fold), and hemopexin (2.4-fold) reliably distinguished DN with macroalbuminuria from DM without albuminuria [71]. Conversely, a significant
decrease in transthyretin (4.3-fold), apolipoprotein A1 (3.2-fold), AMBP (1.6-fold), and retinol-binding plasma protein (1.52-fold) was observed in DN with macroalbuminuria [71]. A model study with selected proteins suggested the significance of cathepsin A, mucin 1, the GM2 ganglioside activator, SPARC-like protein 1, and lysosomal acid phosphatase in the poor prognosis of the early development of DN, as well as in kidney fibrosis [111]. A combination of 408 N-linked glycoprotein, A1AT, and ceruloplasmin was shown to be able to distinguish microalbuminuria and normalbuminuria in DN patients [73]. Urinary haptoglobin and AMBP can differentiate between diabetic patients with and without DN [75]. The increased excretion of 15.8 kDa Clara cell protein CC16 was found to be associated with proximal tubule dysfunction in DM patients with micro- or macroalbuminuria compared to DM patients without albuminuria and healthy controls [76]. The levels of osteopontin and fibronectin were also higher in DN compared to those in DM, and increases in urinary neprilysin and VCAM-1 were observed after losartan treatment in DN [110].

A longitudinal study of type 2 DM revealed an increase in urine transthyretin/prealbumin and the Ig kappa C chain region within 0–5 years of the onset of DM; the appearance of cystatin C and ubiquitin after 5–10 years; and the detection of α-1-acid glycoprotein 1, apolipoprotein A1, AMBP, pigment epithelium-derived factor, and zinc α-2-glycoprotein after 10–20 years [74]. The nonenzymatic glycation of these proteins and their peptides interferes with normal tubular reabsorption and may lead to damage to the proximal tubules and the direct excretion of the proteins into urine.

Overall, the aforementioned DN markers may reflect the processes of tubular atrophy and tubulointerstitial fibrosis, many of which are important for DN prognosis. Zinc-α2-glycoprotein, transthyretin, and AMBP should be especially noted, as their prognostic significance was confirmed in at least two independent studies [71,74,75].

7. Lupus Nephritis

Lupus nephritis (LN) is one of the most common and severe complications of systemic lupus erythematosus and usually appears at least 3-5 years after onset of the disease. The mechanisms of renal glomerulus damage can be found in the deposition of immune complexes or autoantibodies with subsequent complement activation [112]. LN leads to severe kidney damage that advances to end-stage renal disease if not treated adequately. The most important goal for LN treatment is to dynamically assess the degree of renal damage activity since the available activity markers (daily proteinuria, erythrocyturia, complement, and antinuclear antibodies) are not informative. LN patients currently need to undergo several kidney biopsies to monitor LN activity during immunosuppressive therapy to determine where LN treatment should be continued or canceled. In this case, there is a need for highly sensitive and specific LN markers able to predict disease exacerbation or indicate insufficient effectiveness of the therapy.

Only a few potential urinary protein markers specific for LN can be noted (Table 2). A pair of peptides, “3340” and “3980” (m/z), have made it possible to differentiate an acute LN condition from LN remission with 92% sensitivity and 92% specificity prior to any changes in clinical parameters (the urinary protein/creatinine ratio, antibodies to DNA, hematuria, serum creatinine, etc.). Moreover, these peptides were able to predict early relapse and remission [66].

Particular fragments of hepcidin, together with fragments of A1AT and albumin, were found to be more significant than systemic lupus erythematosus renal flare cycle LN in a dynamic study on the urinary proteome [67]. The altered expression of hepcidin 20 might be a marker of renal flare, whereas an increase in hepcidin 25 upon treatment could be used to estimate the effectiveness of therapy [67].

The classifier based on 172 peptides reliably differentiated 92 LN cases from the general CKD group (1180 patients) and identified the protein S100-A9 as another specific LN marker, whose increased level was found to be essential for LN differentiation in combination with
increased levels of collagen peptides and uromodulin, as well as decreased levels of clusterin, \( \beta \)-2-microglobulin, and \( \alpha \)-2-HS-glycoprotein [54].

\( \alpha \)-1-Antichymotrypsin (SERPINA3) is another potential specific LN marker in urine and the only LN marker whose significance was confirmed in two independent studies [68,69]. Together with haptoglobin and retinol-binding protein, SERPINA3 was significantly increased in active LN compared to inactive LN [68]. Moreover, SERPINA3 demonstrated a moderately positive correlation with LN histological activity, which was confirmed via immunohistochemistry [69].

In general, the described LN markers make it possible to assess the activity of the disease and the accumulation of fibrosis in the kidneys, which are very important in clinical practice when managing patients. The increased levels of some proteins may suggest tubular dysfunction during the acute form of the disease [68].

8. Non-Specific Urinary Protein Markers

Uromodulin, collagens, A1AT, and their fragments are the main non-specific urine protein markers that were identified in all the aforementioned nephropathies (Table 2), as well as in many other disorders associated with renal disfunction or proteinuria [17–39]. Uromodulin is a kidney-specific glycosylphosphatidylinositol (GPI)-anchored glycoprotein exclusively produced by the epithelial cells lining the thick ascending limb of the loop of Henle and is a normal component of the urine. Collagen peptides are also normally present in urine and reflect the turnover of the extracellular matrix in kidney tissues. Nevertheless, both usual urine components may indicate pathological changes. Uromodulin may also be a potential biomarker relevant to tubular function and CKD [113]. The level of collagen fragments strongly correlates with the initiation of DN [13,17,19,45,72]; quantitative changes in these fragments in urine were noted 3-5 years prior to the development of macroalbuminuria [19]. Overall, the qualitative composition of the collagen fragments can vary in different nephropathies [45,47,54,72].

Unlike uromodulin and collagen peptides, the appearance of A1AT in urine is always associated with some type of pathology and may reflect podocyte stress [53]. Notably, an increase in urinary A1AT was observed in all the nephropathies reviewed in the present study (Table 2).

In general, the assessment of nonspecific markers in combination with specific markers significantly improved the differentiation of nephropathies. In particular, the levels of six UMOD and A1AT peptides differentiated between proliferative and nonproliferative (including MCD, MN, FSGS, and IgAN) forms of glomerular kidney diseases [58]. Moreover, uromodulin overexpression was shown to predispose one to CKDs such as hypertensive nephropathy and DN [114]. The detection of collagen fragments together with the LG3 fragment of endorepellin is crucial for diagnosing IgAN, as collagen may indicate a more severe disease course with impaired angiogenesis and the rapid development of kidney fibrosis [64]. Estimating the levels of A1AT, uromodulin, transferrin, serum albumin, and \( \alpha \)-1-\( \beta \)-glycoprotein is also important in IgAN, as such levels reflect common pathological processes, including enhanced apoptosis, inflammation, coagulation, and complement activation [45,54,61,62,64,65,72].

9. Conclusions

The research results indicate the great potential of proteomic analysis for the non-invasive diagnosis of kidney diseases, clarification of the leading pathogenetic mechanisms of disease progression, and determination of targets of action for inhibiting disease progression. Unlike kidney biopsy, urine proteomic analysis is safe and reliable, and can be repeated multiple times for the monitoring of disease. The proteomic urinary profile provides valuable information about the leading pathological processes occurring in renal tissues at the time of examination.

The main feature of proteomic analysis is that many of the markers detected in urine are observed as the result of protein penetration from the blood (albumin, retinol-binding
protein, etc.) or as reflections of common pathological processes such as extracellular matrix accumulation (collagens and A1AT), the deposition of immunoglobulin complexes, complement activation, apoptosis, lipid oxidation, and tubular dysfunction (β-2-microglobulin, uromodulin, etc.) with high proteinuria. In this case, it is crucial to assess quantitative changes in these indicators to accurately reflect the process activity and damage severity.

One of the most important goals of urine proteomic analysis in patients with CKD is determining disease-specific biomarkers or their combinations. Proteins extracted for the first time warrant the most attention, as they may reflect the most important pathogenetic stages in disease development. For example, CD44, a marker of activated parietal epithelial cells, may reflect the processes of glomerulosclerosis in MN [50] or IgAN [38] but, at the same time, may also be an essential feature for differentiating FSGS from MCD [52]. DPEP1, primarily identified in FSGS, is thought to reflect TRPC6 activation in podocytes [52]; ubiquitin-60S ribosomal protein L40 (UBA52), which is a marker of cellular stress; or components of the podocyte cytoskeleton that are damaged by antibodies [49,115]. Apolipoproteins, which can play a potential role in FSGS pathogenesis as “permeability factors” [116], as well as proteins whose roles are not yet completely understood, such as lysosome membrane protein-2 and afamin in MN [56,57] and the laminin G-like 3 (LG3) fragment of endorepellin in IgAN [64], may reflect pathological processes and could become targets for new approaches to immunosuppressive or nephroprotective therapy. In addition, positive dynamic changes in the proteomic profile after the designated therapy may help to confirm whether the prescribed medications were chosen correctly and are helping to achieve the desired outcomes. However, despite the validation of the CKD 273 classifier in several studies, there is a need to further develop new panels with increased specificity for specific nephropathies. This seems to be the most important goal for further proteomics research.

Table 2. Potential urine proteome markers in different nephropathies.

| Potential Urine Protein Marker | CKD | FSGS | MCD | MN | IgAN | LN | DN |
|-------------------------------|-----|------|-----|----|------|----|----|
| α1-antitrypsin (A1AT)         | ↑ [45] | ↑ [48,53,54] | ↑ [48,50] | ↑ [54,57,58,82] | ↑ [54,61,62,64,65] | ↑ [67] | ↑ [71,73] |
| Serum albumin                 | ↑ [45] |       | ↑ [64] | ↑ [67] | ↑ [72,74] |
| Hemoglobin                    | ↑ [45] |       | ↓ [54] |
| Fibrinogen α chain            | ↑ [45] | ↓ [54] | ↑ [54] | ↑ [54,58] | ↓ [61] | ↑ [54] | ↓ [54,72] |
| Uromodulin                    | ↑ [45] |       | ↑ [54] |       |       |       |       |
| Na⁺/K⁺-ATPase γ chain         | ↑ [45] |       |       |       |       |       |       |
| Membrane-associated progesterone receptor component 1 | ↑ [45] |       |       |       |       |       |       |
| Collagens                     | ↓ [45] | ↑ [47] | ↑ [54] | ↑ [54] | ↓ [45,62,72] | ↑ [54] | ↓ [72] |
| Cathepsin D                   | ↑ [47] |       |       |       |       |       |       |
| MMP-2                         | ↑ [47] |       |       |       |       |       |       |
| MMP-14                        | ↑ [47] |       |       |       |       |       |       |
| Collagenase 3                 | ↑ [47] |       |       |       |       |       |       |
| α2-HS-glycoprotein            | ↑ [47] |       |       |       | ↑ [54] | ↑ [71] |       |
| Fetuin-A                      | ↑ [47] |       |       |       |       |       |       |
| Cathepsin B                   | ↑ [94] |       |       |       |       |       |       |
| Cathepsin C                   | ↑ [94] |       |       |       |       |       |       |
| Annexin A3                    | ↑ [94] |       |       |       |       |       |       |
| Potential Urine Protein Marker | CKD | FSGS | MCD | MN | IgAN | LN | DN |
|-------------------------------|-----|------|-----|----|------|----|----|
| Transferrin                   |     | ↑ [48] |     |     |      |    |    |
| Histatin-3                    |     | ↑ [48] |     |     |      |    |    |
| 39S ribosomal protein L17     |     | ↓ [48] |     |     | ↑ [48] |    |    |
| (FSGS/MCD)                    |     |      |     |     |      |    |    |
| Calretinin (FSGS/MCD)         |     | ↑ [48] |     |     | ↓ [48] |    |    |
| UBA52 (FSGS/MCD)              |     | ↑ [49] |     |     | ↓ [49] |    |    |
| Cadherin-like 26              |     | ↑ [50] |     |     |      |    |    |
| RNase A Family 1              |     | ↑ [50] |     |     |      |    |    |
| DIS3-like exonuclease 1       |     | ↑ [50] |     |     |      |    |    |
| CD14                          |     | ↑ [50] |     |     |      |    |    |
| Complement C9                 |     | ↑ [50] |     |     | ↑ [59] |    |    |
| Apolipoprotein A1             |     | ↑ [51] |     |     |      |    |    |
| Matrix-remodeling protein 8   |     | ↑ [51] |     |     |      |    |    |
| Dipeptidase 1 (DPEP1)         |     | ↓ [52] |     |     |      |    |    |
| CD59                          |     | ↑ [52] |     |     |      |    |    |
| CD44                          |     | ↑ [52] |     |     | ↑ [50] |    |    |
| Insulin-like growth factor-binding protein 7 |     | ↑ [52] |     |     |      |    |    |
| Roundabout homolog 4          |     | ↑ [52] |     |     |      |    |    |
| Clusterin                     |     | ↓ [54] |     |     | ↓ [54] |    |    |
| Polymeric immunoglobulin receptor |     | ↓ [54] |     |     |      |    |    |
| Golgi-associated olfactory signaling regulator |     | ↓ [54] |     |     |      |    |    |
| Apolipoprotein C-IV           |     | ↑ [54] |     |     | ↑ [54] |    |    |
| β-2-microglobulin             |     | ↓ [54] |     |     | ↑ [54] |    |    |
| Complement C3                 |     | ↓ [54] |     |     | ↑ [63] |    |    |
| Retinol-binding protein 4     |     | ↓ [54] |     |     | ↑ [117] |    |    |
| SH3 domain-binding glutamic acid-rich-like protein 3 |     | [55] |     |     | [55] |    |    |
| Thyroxine-binding globulin    |     | ↑ [50] |     |     |      |    |    |
| (SERPIN A7)                   |     |      |     |     |      |    |    |
| Lysosome membrane protein-2   |     | ↑ [105] |     |     |      |    |    |
| Afamin                        |     | ↑ [55,57,101] |     |     |      |    |    |
| α-1B-glycoprotein             |     | ↑ [54] |     |     | ↑ [64] |    |    |
| Plasminogen                   |     | ↑ [54] |     |     |      |    |    |
| Zinc finger protein ZFPM2     |     | ↓ [54] |     |     |      |    |    |
| E1A-binding protein           |     | ↓ [54] |     |     |      |    |    |
| Microtubule-associated protein tauAP-3 complex subunit delta-1 |     | ↓ [54] |     |     |      |    |    |
| LDB3                          |     | ↑ [100] |     |     |      |    |    |
| PDLI5                         |     | ↑ [100] |     |     |      |    |    |
| Potential Urine Protein Marker | CKD | FSGS | MCD | MN | IgAN | LN | DN |
|-------------------------------|-----|------|-----|----|------|----|----|
| Ig kappa chain C region       | ↑   | ↑    |     |    | ↑    |    |    |
| Cytoskeletal keratins type 1 (10) and type 2 (1, 5) | ↑   |       |     |    |       |    |    |
| ICAM1                         | ↑   |       |     |    |       |    |    |
| Metalloproteinase inhibitor 1 | ↑   |       |     |    |       |    |    |
| Antitrombin III               | ↑   | ↑    |     |    | ↑    |    |    |
| Adiponectin                   | ↑   |       |     |    |       |    |    |
| α-2-macroglobulin             | ↑   |       |     |    |       |    |    |
| Ceruloplasmin                 | ↑   | ↑    |     |    |       |    |    |
| Complement C4a                | ↑   |       |     |    |       |    |    |
| Haptoglobin                   | ↑   | ↑    |     |    | ↑    |    |    |
| Prothrombin                   | ↑   |       |     |    |       |    |    |
| LG3 fragment of endorepellin | ↓   |       |     |    |       |    |    |
| Glycoprotein 2                | ↑   |       |     |    |       |    |    |
| Vasorin                       | ↑   |       |     |    |       |    |    |
| Epidermal growth factor       | ↑   |       |     |    |       |    |    |
| CMRF35-like molecule 9        | ↑   |       |     |    |       |    |    |
| Protocadherin                 | ↑   |       |     |    |       |    |    |
| Utreoglobin                   | ↑   |       |     |    |       |    |    |
| Dipeptidyl peptidase IV       | ↑   |       |     |    |       |    |    |
| NHL repeat-containing protein 3 | ↑   |       |     |    |       |    |    |
| SLAM family member 5 (CD84)   | ↑   |       |     |    |       |    |    |
| Aminopeptidase N              | ↓   |       |     |    |       |    |    |
| Fibulin-5                     | ↓   |       |     |    |       |    |    |
| YIP1 family member 3          | ↓   |       |     |    |       |    |    |
| Prasoposin                    | ↓   |       |     |    |       |    |    |
| Osteopontin                   | ↓   | ↑    |     |    |       |    |    |
| Small proline-rich protein 25 | ↑   | ↑    |     |    |       |    |    |
| Leucine-rich repeat-containing protein 25 | ↑   |       |     |    |       |    |    |
| 3340 and 39110 (m/z)          | ↑   |       |     |    |       |    |    |
| Hepcidin                      | ↑   |       |     |    |       |    |    |
| Protein S89-A9                | ↑   |       |     |    |       |    |    |
| α-1 anti-chymotrypsin (SERPINA3) | ↑   |       |     |    |       |    |    |
| Retinol binding protein       | ↑   |       |     |    |       |    |    |
| Zinc-α2-glycoprotein          |       | ↑    |     |    |       |    |    |
| Vitamin D-binding protein     | ↑   |       |     |    |       |    |    |
| Calgranulin B                 | ↑   |       |     |    |       |    |    |
| Hemopexin                     | ↑   |       |     |    |       |    |    |
| Transthyretin                 |       | ↓    |     |    |       |    |    |
Table 2. Cont.

| Potential Urine Protein Marker | Nephropaty | CKD | FSGS | MCD | MN | IgAN | LN | DN |
|-------------------------------|------------|-----|------|-----|----|------|----|----|
| α-1 microglobulin/bikunin precursor (AMBP) | ↓ [71] | ↑ [74,75] |
| 408 N-linked glycoproteins | ↑ [73] |
| Cystatin C | ↑ [74] |
| Ubiquitin | ↑ [74] |
| α-1-acid glycoprotein 1 | ↑ [74] |
| Pigment epithelium-derived factor | ↑ [74] |
| Clara cell protein CC16 | ↑ [76] |
| Fibronectin | ↑ [110] |

The gray background indicates the most potential markers identified for specific nephropathy in at least 2 studies.

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