UNIVERTISTY OF MEDICINE AND PHARMACY

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DOCTORAL SCHOOL

FIELD: MEDICINE

THE ROLE OF URINARY BIOMARKERS IN THE EARLY DIAGNOSIS AND TREATMENT OF GLOMERULONEPHRITIS

SUMMARY

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2024

Cuprins

Introduction

Glomerulopathies are anatomo-clinical conditions that primarily affect the renal corpuscles, with secondary involvement of the tubules, vessels, and interstitium. The incidence of primary glomerulonephritis is estimated at 57 cases per 100,000 inhabitants in the USA, with a mortality rate 2.7 times higher than in the general population.[1] Although relatively rare, glomerulonephritis is the third leading cause of chronic kidney disease (CKD) in the USA.[2] The term glomerulopathies encompasses various conditions with different progressions and specific treatments. Currently, renal biopsy is the gold standard for diagnosing glomerulonephritis. The main prognostic factors include chronicity indices on biopsy, creatinine levels at onset, and response to treatment. Identifying new diagnostic methods and prognostic factors, including urinary biomarkers, is an important research direction with the potential to facilitate early diagnosis and prognosis determination in glomerulopathies.

General considerations

1. Urinary Biomarkers – General Overview

In 1998, the National Institutes of Health (NIH) Working Group defined biomarkers as objectively measurable characteristics that can be evaluated as indicators of physiological processes, pathological conditions, or responses to therapeutic interventions. They are used for screening, diagnosis, monitoring, and assessing treatment response.[1] The discovery of a new biomarker involves stages such as discovery, qualification, verification, optimization of laboratory techniques, clinical validation, and commercialization.[3] The use of urinary biomarkers has a long history, with the earliest records dating back to before our era. Today, albuminuria is one of the most frequently used urinary biomarkers, indicating endothelial dysfunction in cardiovascular diseases, diabetic nephropathy, and glomerulonephritis.

Inspired by the success of troponin in the early diagnosis of acute coronary syndrome, researchers aim to identify a specific urinary biomarker for acute kidney injury (AKI), considering the high morbidity and mortality rates associated with this condition. Diagnosis still relies on the increase in serum creatinine, and treatment, often delayed, focuses on supporting vital functions,

contributing to the high morbidity and mortality rates associated with AKI. Serum creatinine, used to calculate the glomerular filtration rate, is not an ideal biomarker because its level can be influenced by factors such as age, sex, muscle mass, diet, medications, and hydration status. In AKI, the rise in serum creatinine typically occurs late, after more than 50% of nephrons have been destroyed, and it does not differentiate between types of kidney damage. Thus, early identification of AKI before the development of azotemia is essential to reduce morbidity and mortality.

1. Should be relevant to the histopathological changes of the condition being researched
2. High sensitivity
3. High specificity
4. Non-invasive
5. Applicable across different races, genders, and geographical regions
6. Easily detectable in urine or blood
7. Quick to measure
8. Low cost
9. Rapidly increase in response to kidney injury
10. Able to identify the primary location of the injury (proximal tubule, distal tubule, interstitium, or vessel)
11. Able to discriminate between acute or chronic kidney damage (AKI, CKD, or acute exacerbation of CKD)
12. Able to identify the cause (sepsis, toxic, ischemic, cardiovascular disease, diabetic nephropathy, lupus, combined factors)
13. Able to differentiate subtypes of kidney injury (pre-renal, intrinsic renal, or post-renal obstructive)
14. Role in risk stratification
15. Role in prognosis estimation (predicting dialysis requirement, hospitalization duration, mortality)
16. Monitoring treatment response
17. Stable over time regardless of temperature and pH condition
18. No interference with medications or endogenous substances [3,4,5]

Tabel 1.1 - Characteristics of Ideal Urinary Biomarkers in AKI and CKD

2. Immunity in glomerulonephritis

Glomerulonephritis (GN) are immune-mediated kidney diseases characterized by an imbalance between pro-inflammatory and protective factors. The etiology of GN is usually unknown, except for those induced by infections, medications, or neoplasms. In 2013, GN was the ninth leading cause of death in the USA, according to the Centers for Disease Control and Prevention.[6] Glomerular damage is complex, resulting from the interaction of genetic and environmental factors, and an inadequate immune response triggers pathological manifestations.[7,8] Regardless of the underlying cause, glomerular injury activates a common profibrotic pathway, leading to tubular atrophy, interstitial fibrosis, and loss of nephron mass. The immune response involves both humoral and cellular immune mechanisms, with the formation of immune complexes containing immunoglobulins and complement components, which deposit at the glomerular level or form locally in the presence of the responsible antigen. The antigens may be constituents of the glomerulus, originate from systemic circulation, or be exogenous antigens sequestered at the renal level.

2.1 Inflammatory immune response

The clinical expression of the inflammatory immune response in glomerulonephritis is manifested by dysmorphic hematuria, proteinuria, and a decrease in the glomerular filtration rate. The main inflammatory glomerulonephritis types include IgA nephropathy, lupus nephritis, infection-associated GN, ANCA-positive vasculitis, and membranoproliferative GN. The deposition of immune complexes at the subendothelial, mesangial, or glomerular basement membrane levels triggers the inflammatory immune response. Exceptions include ANCA-positive vasculitis and C3 glomerulopathy, where the immune response occurs without antibody deposition. These processes lead to the recruitment of proinflammatory cells and kidney injury. [9]

The main cells involved in the inflammatory immune response in glomerulonephritis are neutrophils, monocytes/macrophages, T lymphocytes, platelets, and resident renal cells (endothelial cells, mesangial cells, parietal epithelial cells).

Neutrophils play an essential role in the pathogenesis of glomerulonephritis, being present early in kidney biopsies of patients with post-streptococcal GN, MPGN, IgA nephropathy, lupus nephritis, and some forms of RPGN. At the glomerular level, neutrophils interact with endothelial cells and become activated, releasing reactive oxygen species and activating complement, which amplifies inflammation and damages the capillary wall. In severe forms of GN, such as ANCA-positive vasculitis, neutrophils generate extracellular "traps" that cause vascular necrosis.[8]

The recruitment of monocytes at the renal level occurs through interaction with deposited immunoglobulins or chemokines such as MCP-1, MIP-1-alpha, RANTES, and MMP-9. The interaction between MCP-1 and macrophages has been extensively studied and is considered crucial for early diagnosis and treatment guidance in glomerulonephritis.

Macrophages are involved in both humoral and cellular immune responses and are the main inflammatory cells stimulated by T lymphocytes in the absence of antibodies. They produce reactive oxygen species, proteolytic enzymes, and tissue growth factors that stimulate crescent formation and fibrogenesis processes, contributing to glomerulosclerosis. The involvement of macrophages in crescent formation, which is associated with disease severity and poor prognosis, is critical. In an experimental model of Goodpasture's disease, macrophages constituted a significant proportion of the cells forming the crescents, originating from the periglomerular interstitium through Bowman’s capsule rupture.[10,11] Macrophages also contribute to sustaining inflammation and crescent proliferation by releasing growth factors, IL-1, TNF, and TGF-beta. TGF-beta plays a crucial role in disease activity and chronicity, promoting the transition of cellular crescents into fibrocellular and fibrous crescents.

CD 163+ macrophages, a subclass of macrophages and the main CD 163-producing cells, are also involved in the inflammatory processes responsible for the development of glomerulonephritis. Their presence is primarily described in areas of fibrinoid necrosis and crescents. [12,13]

2.2 **Non – inflammatory immune response**

Non-inflammatory immune mechanisms manifest as pure nephrotic syndrome without hematuria or red blood cell casts. The main causes include membranous nephropathy, minimal change nephropathy, and focal segmental glomerulosclerosis, collectively referred to as

podocytopathies. In these conditions, the primary lesion affects podocytes, increasing glomerular permeability. The involved immune mechanisms cause podocyte dysfunction without triggering inflammation and are often associated with T lymphocyte-derived factors, vasoactive agents, membrane attack complex, and cytokines such as IL-13 and CLC-1.[8]

In minimal change nephropathy (MCN), CD80 and ANGPTL-4 molecules play a crucial role. CD80, activated by bacterial or viral antigens, disrupts podocyte structure, leading to proteinuria. ANGPTL-4, a glycoprotein excessively produced by podocytes, increases glomerular permeability and induces proteinuria.[8,14] In focal segmental glomerulosclerosis (FSGS), factors such as CLC-1 and suPAR are implicated in disease pathogenesis, although the role of suPAR remains controversial.[8,14] In membranous nephropathy (MN), the immune response is triggered by autoantibodies binding to podocyte antigens, activating complement, and causing podocyte damage. The main identified autoantibodies target the phospholipase A2 receptor and thrombospondin. Activation of the membrane attack complex (C5b-9) at the podocyte level contributes to proteinuria by stimulating the production of proteases and cytokines, cytoskeletal reorganization, and podocyte apoptosis. Measuring urinary C5b-9 levels or podocyte excretion could be useful in diagnosing and monitoring treatment in MN.[15,16,17]

3. Urinary biomarkers in glomerulonephritis

3.1 Monocyte chemotactic protein 1

Perhaps the most well-known chemokine is MCP-1. It consists of 76 amino acids, has a molecular weight of approximately 13 kDa, and is derived from a 99-amino acid precursor through proteolytic cleavage. MCP-1 is produced by various cells, including endothelial cells, fibroblasts, smooth muscle cells, mesangial cells, and monocytes/macrophages, the latter being the major source of MCP-1.[18] The main stimuli for MCP-1 production include pro-inflammatory cytokines (IL-1, IL-4, TNF-α, IFN-γ), growth factors (macrophage colony-stimulating factor [M-CSF], granulocyte-macrophage colony-stimulating factor [GM-CSF], PDGF, VEGF),

lipopolysaccharides, reactive oxygen species, oxidized LDL molecules, and immune complexes. Inhibitors of MCP-1 expression include TGF-β, retinoic acid, glucocorticoids, and estrogen.

Urinary expression of MCP-1 has been extensively studied in patients with renal vasculitis, a group of conditions with a poor renal and overall prognosis. Experimental studies from the late 20th century showed that urinary levels of MCP-1 correlate with macrophage infiltration in the glomeruli.[19] In ANCA-positive vasculitis, the main factors for vital prognosis include target organ involvement and elevated serum ANCA levels, while for renal prognosis, the need for dialysis at diagnosis, response to induction therapy, and frequency of relapses are crucial factors. Histopathological changes with prognostic value include interstitial fibrosis and glomerulosclerosis, though study results remain controversial.

Recent studies focus on the diagnostic and prognostic value of urinary MCP-1. Research has shown that elevated urinary MCP-1 levels in patients with active renal vasculitis correlate with ANCA titer and BVAS score but not with proteinuria. Urinary MCP-1 has been identified as a marker of poor prognosis, possibly reflecting long-term subclinical inflammation or tubulointerstitial damage.[20]

IgA nephropathy is the most common glomerulopathy, with a variable course ranging from mild forms to chronic renal failure, affecting 25-40% of patients. The main prognostic factors include proteinuria, blood pressure, and serum creatinine. The MEST-Oxford score, based on histopathological changes, provides additional prognostic information. Mesangial C4d deposition is a risk factor for the progression of renal disease. Urinary excretion of MCP-1 in patients with IgA nephropathy correlates with interstitial fibrosis and glomerular sclerosis, serving as a potential marker of tubulointerstitial inflammation and disease severity.[21]

MCP-1 is primarily produced by tubular epithelial and interstitial cells in patients with IgA nephropathy, and proteinuria plays a crucial role in disease progression. MCP-1 is elevated in both IgA nephropathy and diabetic nephropathy, being linked to proteinuria and tubulointerstitial inflammatory infiltration. [22] However, more studies with a larger number of patients are needed to correlate these findings with renal biopsies. [22]

Similar results were obtained in studies conducted on patients with membranous nephropathy. In a study published in 2004 involving 30 patients with membranous nephropathy who underwent renal biopsy, Yoshimoto et al. showed that interstitial inflammatory infiltration with CD68-positive macrophages at the time of diagnosis was associated with progression to end-

stage renal disease. These interstitial CD68-positive macrophages express MCP-1. Similar to studies conducted on patients with IgA nephropathy and proteinuria, interstitial MCP-1 expression is associated with the progression of tubulointerstitial lesions and unfavorable outcomes. Additionally, the study suggests that cells expressing the CCR-2 receptor, the primary receptor for MCP-1, are found in the renal interstitium alongside MCP-1 expression. The CCR-2 receptor is an independent risk factor for progression to end-stage renal disease.[23]

3.2 Urinary soluble CD 163

CD163 is a type I transmembrane protein with a molecular weight of 130 kDa, found on M2c macrophages responsible for the reparative processes of inflammation. It belongs to the scavenger receptor superfamily type B with a high cysteine content and is responsible for interacting with hemoglobin-haptoglobin complexes. CD163 is found exclusively on monocytes-macrophages, particularly on M2c macrophages. The expression of CD163 is stimulated by glucocorticoids, IL-6, IL-10, heme/hemoglobin, and oxidative stress, while IL-4, lipopolysaccharides, TNF-alpha, interferon-γ, CXC-chemokine ligand 4, and granulocyte-macrophage colony-stimulating factor inhibit CD163 receptor expression.[24,25]

The activity of soluble urinary CD163 has been primarily studied in patients with ANCA-positive vasculitis and lupus nephritis, correlating with fibrinoid necrosis lesions and the presence of cellular crescents, accurately distinguishing between patients with active renal disease and those in remission. Therefore, soluble urinary CD163 is a serious candidate for the early diagnosis of patients with ANCA vasculitis with renal involvement.

Few studies have investigated the role of soluble urinary CD163 in patients with IgA nephropathy. Results showed a direct correlation with 24-hour proteinuria levels and serum creatinine, and an inverse correlation with eGFR, hemoglobin, and serum albumin levels. Additionally, the concentration of soluble urinary CD163 was analyzed in relation to the MEST score. Patients with mesangial or endocapillary hypercellularity, glomerulosclerosis, or tubulointerstitial lesions, as well as those with crescents in more than 25% of glomeruli, showed increased urinary concentrations of soluble CD163. Unfortunately, the increase in soluble urinary CD163 cannot distinguish between these changes, as it is elevated in both acute and chronic lesions. However, the study supports an association, particularly with the severity of tubulointerstitial lesions.[26]

3.3 Neutrofil gelatinase-associated lipocalin urinară

NGAL (Neutrophil Gelatinase-Associated Lipocalin) is a protein with a molecular weight of 25 kDa and is part of the lipocalin family. It is also known as lipocalin 2 or siderocalin.

NGAL appears in urine through two main pathways. Firstly, NGAL from the blood is filtered at the glomerular level and then reabsorbed by endocytosis in the proximal tubules, which makes urinary NGAL less influenced by extrarenal synthesis. Plasma NGAL is produced in other organs (such as the lungs and liver), as well as in circulating neutrophils and macrophages under specific stimulation conditions, which reduces its predictive capacity for renal conditions compared to urinary NGAL. In the presence of tubular injury, the NGAL gene in tubular epithelial cells is stimulated, leading to an increase in urinary NGAL concentration.[27]

In the kidneys, NGAL plays a protective role by binding iron and transferring it to proximal tubular epithelial cells. It is also involved in the innate immune system by inhibiting bacterial growth through binding to the siderophore-iron complex, which is a major source of iron in the kidneys. NGAL is also involved in the early stages of neutrophil maturation.[28]

Although NGAL appears to be an essential biomarker for the early diagnosis of acute kidney injury (AKI), studies also support its importance in diagnosing and monitoring glomerulonephritis. Average urinary NGAL levels in patients with glomerulonephritis are five times higher than those in healthy individuals and 30 times lower than in patients with AKI. The presence of tubular lesions associated with glomerular changes leads to NGAL secretion. Proteinuria has toxic effects on tubular epithelial cells, stimulating local NGAL secretion.[29]

In ANCA-associated vasculitis, NGAL is synthesized both in neutrophils and monocytes as well as in tubular cells. In membranous nephropathy (MN), urinary NGAL concentration is higher in nephrotic patients compared to those with subnephrotic proteinuria.[30] Similar results are observed in other glomerular nephropathies.

Urinary NGAL is a useful marker for early detection of tubular injury and in IgA nephropathy. Although there are no well-established prognostic markers for this disease, urinary NGAL may fill this gap. Studies show that urinary NGAL correlates with early tubulointerstitial lesions (stages II and III) but does not reflect advanced damage. For example, a 2007 study showed

that while urinary NGAL is an early indicator, it does not correlate with advanced stages of injury.[31]

Special section

4. Working Hypothesis and General Objectives

Glomerulopathies are conditions that primarily affect the renal corpuscles, with secondary impact on the tubules, vessels, and interstitium. In the US, primary glomerulonephritis has an incidence of 57 cases per 100,000 residents and is the third leading cause of chronic kidney disease (CKD).[2] Diagnosis is mainly made through renal biopsy, but there are associated risks. Recent research indicates that urinary biomarkers such as MCP-1, soluble CD163, and NGAL are useful in diagnosing and monitoring glomerulonephritis, showing significant predictive value. The study aims to evaluate the role of these biomarkers, correlate their values with histopathological changes, clinical parameters, and chosen therapeutic strategies, and develop a personalized diagnostic and treatment protocol based on these biomarkers.

5. General Research Methodology

Pacients

We conducted a retrospective, observational, single-center study involving a group of 70 volunteers divided into three groups: Group A – 42 patients with glomerular pathology (IgA nephropathy, pauci-immune glomerulonephritis, membranoproliferative glomerulonephritis, minimal change glomerular nephropathy, membranous nephropathy, focal and segmental glomerulosclerosis); Group B – 8 patients with renal impairment of hypertensive nephropathy/ thin basement membrane disease; and Group C – the control group, consisting of 20 healthy volunteers. The patients were admitted between 2018 and 2024 at the Dr. Carol Davila Clinical Hospital of Nephrology, Bucharest, Romania.

Patient enrollment was carried out after signing the informed consent form, and all study procedures were conducted in accordance with the principles of the Helsinki Declaration. The study was approved by the Ethics Committee of the Dr. Carol Davila Clinical Hospital of Nephrology. Clinical and paraclinical data collected at the time of study inclusion were obtained from documents related to the patients' medical history (previous discharge summaries, medical tests, outpatient consultations, etc.) and observation sheets from the hospitalization period.

All patients underwent renal biopsy as a diagnostic method. Sterile urine samples were collected on the morning of the renal biopsy from the midstream into sterile containers. The samples were centrifuged and stored at -20 degrees Celsius until processed.

Inclusion Criteria:

- Patients with a diagnosis of glomerulonephritis based on renal biopsy results.
- Complete clinical and laboratory data.
- Age > 18 years.

Exclusion Criteria:

- Patients with incomplete clinical or laboratory data.
- Patients with the following histopathological diagnoses: diabetic nephropathy, renal amyloidosis, nephropathy with casts, lupus nephritis.
- Patients with positive urine cultures (the presence of bacteria in the urine alters urinary MCP-1 concentrations).

Renal Biopsy Procedure:

The renal biopsy was performed under local anesthesia and ultrasound guidance, following the patient’s informed consent. It was necessary for the diagnosis of the patient's condition. The obtained samples were examined using optical microscopy, electron microscopy, and immunofluorescence to detect the following reactants: albumin, IgA, IgG, IgM, C1q, C3c, kappa and lambda light chains.

The following histopathological data were collected after the biopsy interpretation: date of the biopsy, number of affected glomeruli, percentage of glomeruli with sclerosis, percentage of

tubular atrophy and interstitial fibrosis, presence of crescents, and presence of endocapillary proliferation.

Urine Sample Processing:

Sterile urine samples were collected on the morning of the renal biopsy from the midstream into sterile containers. The samples were centrifuged and stored at -20 degrees Celsius until processed. Urinary biomarkers (MCP-1, soluble CD163, and urinary NGAL) were analyzed from the same urine samples using ELISA tests (Quantikine R&D Systems, Minneapolis, USA).

Statistical Analysis:

- Descriptive statistics will be used to summarize the data.
- Two means will be compared using the t-test for independent samples.
- Correlations between urinary MCP-1 levels and clinical/historical parameters will be analyzed using Pearson or Spearman correlation coefficients, depending on data distribution.
- Multiple linear regression analysis will be performed to identify the ability of MCP-1, soluble CD163, and urinary NGAL to predict histopathological outcomes.
- Statistical significance will be established at $p < 0.05$.

6. Study 1: The Role of MCP-1 in the Early Diagnosis and Treatment of Glomerulonephritis

Processing of Samples for Determining Urinary MCP-1 Concentration

Urine samples were processed using ELISA tests according to the manufacturer's instructions (Quantikine R&D Systems, Minneapolis, USA).

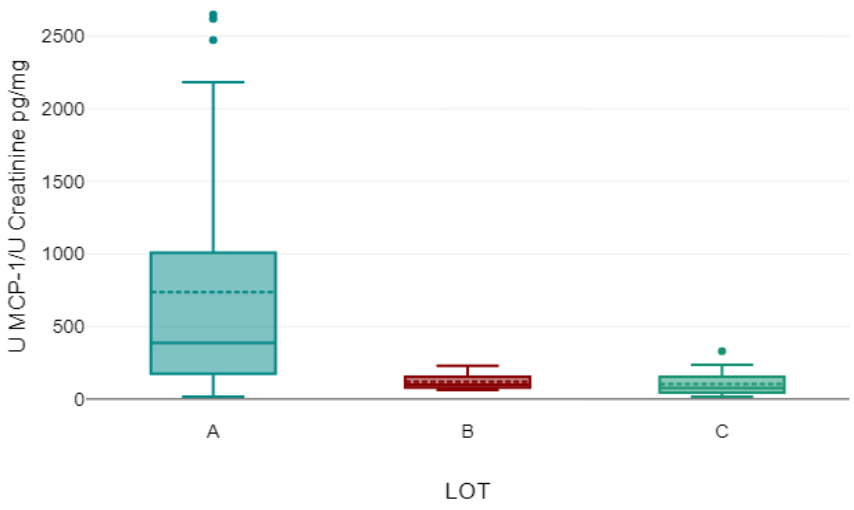

Fig 6.1 Mean Urinary MCP-1 Concentration Normalized to Urinary Creatinine Among the 3 Groups

	n	Mean MCP-1(pg/mg creatinine)	Standard deviation
MCD	9	827.31	943.77
IgA nephropathy	15	617.54	742.65
Membranous nephropathy	10	792.4	931.68
Pauci-imune GN	4	**1118.43**	368.66
Membranoproliferative GN	3	493.94	415.66
GSFS	1	335.34	NaN
Thin basement membrane disease	6	120.59	60.83

	n	Mean MCP-1(pg/mg creatinine)	Standard deviation
Hypertensive nephropaty	2	115.04	71.45
control	20	104.24	85.18
Total	70	485.17	674.96

Statistical analysis revealed a significant difference in urinary MCP-1 concentration among the three patient groups. Patients in group A had a mean concentration of 736.28 ± 775.4 pg/mg creatinine, which was significantly higher compared to patients in group B (119.2 ± 58.13 pg/mg creatinine) and patients in group C (104.24 ± 85.18 pg/mg creatinine), with $p < 0.001$.

Tabel 6.1 Distribution of Patients by Renal Biopsy Results and Mean Urinary MCP-1 Value

The average value of urinary MCP-1 concentration was higher in patients who exhibited crescents on biopsy compared to those without crescents (878.59 ± 413.49 vs. 513.24 ± 695.93), but the difference was not statistically significant, $p = 0.121$. Urinary MCP-1 concentration appears to be associated with the severity of interstitial inflammatory infiltrate, which was statistically significant. Patients without inflammatory infiltrate have much lower urinary MCP-1 levels. Other histopathological changes (endocapillary and mesangial proliferation) do not show statistically significant associations with urinary MCP-1 levels.

The initial treatment of patients in group A was as follows (treatment was administered prior to the PBR result):

			n	U MCP-1/U creatinine pg/mg	P value
Corticosteroid Therapy	Yes		32	874.09 ± 803.73pg/mg	**P=0.038**
	No		10	295.3 ± 479.96 pg/mg	
Cyclophosphamide	Yes		14	803.33 ± 881.09 pg/mg	P=0.7
	No		18	888.03 ± 613.72 pg/mg	

7. Study 2: The Role of Soluble Urinary CD163 in the Early Diagnosis and Treatment of Patients with Glomerulonephritis

Processing of Samples for Soluble Urinary CD163

To determine the concentration of soluble urinary CD163, we used ELISA tests (Quantikinine R&D Systems Minneapolis, USA). Although the kit was not validated for urinary samples, similar kits from the same manufacturer have been used in other clinical studies with different sample dilutions. We diluted the urine samples 1:2 and then proceeded according to the manufacturer's instructions.

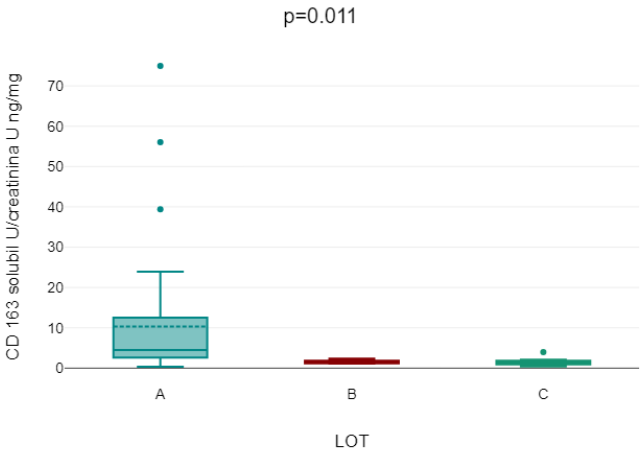

Fig. 7.1 Average Concentration of Soluble Urinary CD163 Normalized to Creatinine in the Three Groups

The statistical analysis revealed a significant difference in the concentration of soluble urinary CD163 across the three patient groups. Patients in group A had an average concentration of soluble urinary CD163/urinary creatinine of 10.36 ± 14.89 pg/mg creatinine, which was significantly higher compared to patients in group B (1.61 ± 0.45 pg/mg creatinine) and group C (1.47 ± 0.79 pg/mg creatinine), p=0.01.

		n	CD 163 urinary soluble/creatinine ng/mg	Standard deviation
CD 163 urinary soluble / urinary creatinine ng/mg	control	20	1.47	0.79
	IgA nephropathy	15	4.2	4.14
	Membranous nephropathy	10	**16.56**	18.11
	MCD	9	8.83	6.32
	Thin basement membrane disease	6	1.75	0.44
	Pauci-imune GN	4	10.84	9.36
	Membrano-proliferative GN	3	**26.45**	42.02
	Hypertensive nephropathy	2	1.19	0.07
	GSFS	1	4.26	NaN

Table 7.1 Distribution of Patients by Renal Biopsy Result and Mean Concentration of Soluble Urinary CD163 Normalized to Creatinine

In the subgroup of patients with nephritic syndrome/nephritic-nephrotic syndrome (31 patients—8 patients from group B, the rest from group A), the concentration of soluble urinary CD163 is higher in patients with crescents on renal biopsy results (21.28 ± 27.34 ng/mg creatinine) compared to patients without crescents (2.9 ± 3.09 ng/mg creatinine), with the difference being statistically significant, p=0.002. Elevated urinary levels of soluble CD163 were observed in patients with active lesions of crescent formation and endocapillary proliferation (statistically significant results). Mesangial proliferation lesions do not alter urinary levels of

soluble CD163, indicating that the source of macrophages is more likely related to crescents or endocapillary proliferation. The severity of interstitial inflammatory infiltrate does not correlate with urinary soluble CD163 levels, but its absence is associated with the lowest urinary concentrations, though this difference is not statistically significant.

		Numar pacienti	CD163 urinary soluble/U creatinine ng/mg	P value
Corticosteroid Therapy	da	32	12.56 ± 16.47pg/mg	P=0.087
	nu	10	3.32 ± 2.14 pg/mg	
Cyclophosphamide	da	14	14.56 ± 20.22 pg/mg	P=0.17
	nu	18	7.29 ± 6.44 pg/mg	

Table 7.3: Concentration of Soluble Urinary CD163 Normalized to Urinary Creatinine Based on Induction Treatment

8. Study 3: The Role of Urinary NGAL in the Early Diagnosis and Treatment of Patients with Glomerulonephritis

9.

Processing of NGAL Samples

Urine samples were processed using ELISA tests according to the manufacturer's instructions (Quantikinine R&D Systems, Minneapolis, USA).

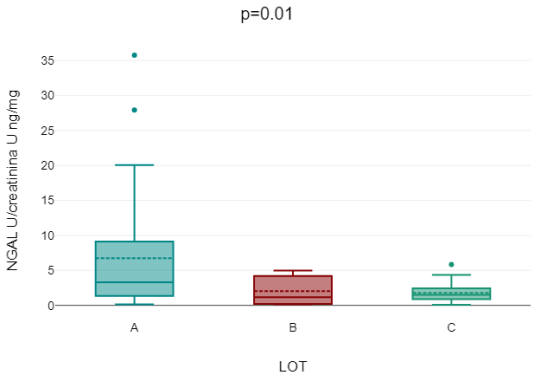

Fig. 8.1 Average Urinary NGAL Concentration Normalized to Creatinine in the Three Patient Groups

The statistical analysis revealed higher urinary NGAL values in patients from group A compared to those in group B or group C (6.73 ± 8.02 ng/mg creatinine versus 2.05 ± 2.1 ng/mg creatinine, and 1.8 ± 1.5 ng/mg creatinine, respectively), with p=0.01.

	n	NGAL urinary/urinary creatinine ng/mg	Standard deviation
control	20	1.8	1.5
IgA nephropathy	15	4.12	3.61
Membranous nephropathy	10	8.58	11.3
MCD	9	7.96	9.5
Thin basement membrane disease	6	2.46	2.28
Pauci-imune GN	4	7.47	8.74
Mempranoproliferative GN	3	6.45	8.65
Hypertensive GN	2	0.81	0.95
GSFS	1	14.1	NaN

Table 8.1 Distribution of patients by renal biopsy result and average urinary NGAL value

In the subgroup of patients with nephritic syndrome/nephritic-nephrotic syndrome (31 patients – 8 patients from group B, the rest from group A) – the urinary NGAL concentration is higher in patients who present crescents in the renal biopsy result (8.56 ± 8.55 ng/mg creatinine) compared to patients without crescents (3.1 ± 2.78 ng/mg creatinine), with a statistically significant difference, $p<0.01$, as well as in those with endocapillary proliferation (8.43 ± 8.23 ng/mg creatinine compared to 3.13 ± 3.03 ng/mg creatinine), p=0.013. The presence of significant interstitial inflammatory infiltrate correlates with higher urinary NGAL values.

Although patients who received corticosteroid therapy at diagnosis (before the biopsy result was received) had higher values compared to those who were not treated, the difference is not statistically significant.

		n	Urinary NGAL/urinary creatinine ng/mg	P value
Corticosteroid Therapy	da	32	7.73 ± 8.88 ng/mg	P=0.149
	nu	10	3.52 ± 2.58 ng/mg	
Cyclophosphamide	da	14	7.12 ± 10.28 ng/mg	P=0.8

Tabel 8.3 The average values of urinary NGAL normalized to urinary creatinine based on initial treatment

1. Conclusions and Special Contributions

1.1 Conclusions

Research conducted so far has aimed to identify diagnostic and prognostic correlations between different urinary biomarkers and various types of glomerulonephritis.

This study covered a wide range of renal pathologies: those associated with nephritic syndrome (pauci-immune GN, membranoproliferative GN, IgA nephropathy), and those associated with nephrotic syndrome (minimal change disease, membranous nephropathy, focal segmental glomerulosclerosis) – Group A of patients. Chronic renal pathologies that progress slowly and do not require immunosuppressive treatment (thin basement membrane disease, hypertensive nephropathy) were included in Group B.

The main objectives of this study were to correlate urinary concentrations of MCP-1, soluble urinary CD163, and NGAL with different types of glomerulonephritis, as well as with the severity of active/chronic lesions identified by renal biopsy. We aimed to find correlations with the following types of active lesions: the presence of crescents, endothelial proliferation, mesangial

proliferation, and interstitial inflammatory infiltrate, as well as with the chronicity score calculated based on the severity of glomerulosclerosis and tubular atrophy/interstitial fibrosis. Among clinical parameters, we assessed correlations with creatinine levels, proteinuria, and the presence/absence of nephrotic syndrome at onset. The literature does not provide established cut-off values for MCP-1 and soluble urinary CD163, with some studies attempting to determine appropriate cut-off values for each biomarker independently.

Patients in Group A showed elevated values for all three urinary biomarkers compared to patients in Group B or the control group, with statistically significant differences. Although we anticipated higher values in the control group, the study results showed similar urinary concentrations of the three biomarkers between Group B patients and the control group: MCP-1 (119.2 ± 58.13 pg/mg creatinine vs. 104.24 ± 85.18 pg/mg creatinine), soluble urinary CD163 (1.61 ± 0.45 ng/mg creatinine vs. 1.47 ± 0.79 ng/mg creatinine), and NGAL (2.05 ± 2.1 ng/mg creatinine vs. 1.8 ± 1.5 ng/mg creatinine). This aspect may indicate the utility of biomarkers in differentiating patients who require renal biopsy, especially in at-risk categories (e.g., patients with a single functioning kidney, those at high risk of bleeding, etc.).

Our study confirms previous research showing elevated urinary MCP-1 levels in patients with active renal vasculitis. Although the highest levels were observed in patients with pauci-immune glomerulonephritis, the differences were not statistically significant. In patients with renal crescents, urinary MCP-1 levels were higher but did not reach statistical significance, similar to Takashi Wada's findings.[32] In contrast to earlier studies, patients with nephrotic syndrome in our study had elevated urinary MCP-1 levels, suggesting that the source might be tubular epithelial cells affected by increased proteinuria. However, further studies are needed to confirm these findings.

Urinary MCP-1 concentrations were associated with the severity of interstitial inflammatory infiltrate described on biopsy in the patients included in our study. Consistent with previous research, MCP-1 is produced by infiltrative macrophages in renal lesions and by resident renal cells (tubular epithelial cells, parietal cells) but does not differentiate between various types of renal lesions. Proteinuria, an important factor in renal disease progression, can induce MCP-1 production at the level of tubular epithelial cells, explaining the elevated urinary MCP-1 levels in nephrotic syndrome patients included in the study, who did not have active lesions or high chronicity scores. Studies suggest that MCP-1 could be an early marker of tubular damage

associated with nephrotic syndrome and that proteinuria itself stimulates MCP-1 synthesis, contributing to the progression of inflammation and renal damage.

The study showed that patients with glomerulonephritis who had elevated urinary MCP-1 levels were treated with corticosteroids from the onset, before histopathological confirmation, suggesting that urinary MCP-1 is associated with more aggressive forms of the disease. Elevated MCP-1 levels may help identify patients who require renal biopsy and intensive treatment. However, the study did not track patients long-term to evaluate the prognostic value of MCP-1 in treatment response and renal disease progression.

While MCP-1 can be sourced from various types of cells (macrophages, tubular epithelial, mesangial), soluble CD163 is secreted specifically by M2c macrophages. Studies have shown the presence of M2c macrophages primarily in fibrinoid necrosis lesions and also in cellular crescents, suggesting their involvement in the pathogenesis of glomerular lesions in ANCA-positive vasculitis.[33]

The study results demonstrated increased urinary CD163 concentrations in patients with crescents (21.28 ± 27.34 pg/mg creatinine vs. 2.9 ± 3.09, $p < 0.001$) and in those with endocapillary proliferation lesions (18.63 ± 28.84 ng/mg creatinine vs. 3.53 ± 3.76 ng/mg creatinine, $p = 0.012$). The inflammatory infiltrate and mesangial proliferation did not affect the concentration of this biomarker, which supports the findings of the study published by Lei Zhao et al. Unlike MCP-1, which is produced interstitially, CD-163 production depends on the presence of M2c macrophages.[33]

The study found that the highest urinary soluble CD-163 values were observed in patients with membranoproliferative nephropathy, membranous nephropathy, and pauci-immune glomerulonephritis. However, values were similar across different types of glomerulonephritis, suggesting that urinary soluble CD-163 may not be a distinctive biomarker for these conditions. Elevated urinary CD-163 in patients with membranous nephropathy indicates the role of M2c macrophages in the pathogenesis of this pathology. Additionally, the measurement methodology and the small patient sample size may limit the conclusions. Future studies with larger and more homogeneous groups, as well as the investigation of other biomarkers, are needed to clarify the role of urinary soluble CD-163.

Urinary concentrations of soluble CD163 do not correlate with baseline creatinine levels, proteinuria at onset, or the chronicity score calculated from the biopsy. However, they do show a

positive correlation with the presence of active lesions (endocapillary proliferation, crescents), with statistically significant correlations obtained.

Patients with elevated levels of soluble CD163 received more aggressive treatment, including corticosteroids and cyclophosphamide, even before histopathological results were available, although these differences were not statistically significant. Elevated urinary CD163 and MCP-1 levels were associated with the need for more intensive immunosuppressive treatment. These biomarkers could be useful in identifying more severe forms of glomerular disease and guiding early therapeutic decisions, but further studies are needed to validate their clinical and statistical relevance.

The two biomarkers represent two facets of the same inflammatory process—MCP-1 is secreted by leukocytes and renal tubular cells in response to inflammatory factors and has a chemotactic role for macrophages that express CD163.

The renal source of NGAL is production by tubular epithelial cells, which can be stimulated under certain conditions (e.g., significant proteinuria) or by decreased reabsorption in the proximal convoluted tubule, as well as by neutrophils in patients with ANCA vasculitis.

The study results showed higher urinary NGAL values in patients from group A compared to those in group B or the control group (6.73 ± 8.02 ng/mg creatinine vs. 2.05 ± 2.1 ng/mg creatinine and 1.8 ± 1.5 ng/mg creatinine), $p = 0.01$. Urinary NGAL concentration did not correlate with baseline creatinine levels or proteinuria.

Similar to the other two urinary biomarkers, elevated NGAL concentrations were observed in patients with membranous nephropathy (8.58 ± 11.3 ng/mg creatinine), NGLM (7.96 ± 9.5 ng/mg creatinine), pauci-immune GN (7.47 ± 8.74 ng/mg creatinine), and membranoproliferative GN (6.45 ± 8.65 ng/mg creatinine), with results being similar across these four pathologies. In conditions that evolve with nephrotic syndrome, NGAL synthesis is stimulated in tubular epithelial cells by increased proteinuria. Patients with nephrotic syndrome and those with nephritic-nephrotic syndrome had higher urinary NGAL concentrations compared to those with nephritic syndrome.

Statistical analysis based on the presence of active lesions showed that urinary NGAL concentration correlated with the presence of crescents, endocapillary proliferation, and the severity of interstitial inflammatory infiltrate, with statistically significant results. However, it did not correlate with the chronicity score.

Urinary NGAL levels did not correlate with the type of treatment received at onset, although patients who received corticosteroids and/or cyclophosphamide had higher values (7.73 ± 8.88 ng/mg and 7.12 ± 10.28 ng/mg, respectively, compared to 3.52 ± 2.58 ng/mg and 6.4 ± 6.87 ng/mg, respectively).

Pearson correlation results indicate a positive correlation between urinary levels of MCP-1, NGAL, and soluble CD163, when analyzed in pairs.

Logistic regression analysis showed that the association between serum creatinine, serum albumin, proteinuria, and urinary concentrations of the three biomarkers can predict histopathological outcomes (NGLM or IgA nephropathy). However, when analyzed separately, none of the variables alone had sufficient statistical power.

Further studies with larger patient cohorts are needed to confirm the diagnostic and prognostic value of these three urinary biomarkers in patients with glomerulonephritis and to establish normal reference values.

9.2 Personal Contributions

The study demonstrated the association of urinary levels of biomarkers MCP-1, NGAL, and soluble CD163 with glomerular-type renal impairment. These biomarkers can assist in triaging patients with nephritic syndrome who would benefit from a renal biopsy. MCP-1 correlated with the severity of interstitial inflammatory infiltrate, soluble CD163 with the presence of crescents and endocapillary proliferation, and NGAL with all three active lesions. However, these biomarkers did not successfully differentiate between different types of glomerulonephritis.

Additionally, we encountered difficulties in finding validated ELISA kits for measuring soluble CD163 in urine. Additionally, the data showed elevated levels of soluble CD163 in patients with nephrotic syndrome, suggesting the involvement of M2c macrophages in the pathogenesis of these conditions.

Given the high costs of reagents, these biomarkers cannot currently be used routinely. Further studies with larger cohorts are required to establish normal values and the role of these biomarkers in the diagnosis and treatment of glomerulonephritis.

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