Ukrainian Journal of Nephrology and Dialysis
Scientific and Practical, Medical Journal

Founder:
- National Kidney Foundation of Ukraine

ISSN 2304-0238;
eISSN 2616-7352

Journal homepage: https://ukrjnd.com.ua

Research article

doi: 10.31450/ukrjnd.4(88).2025.03

I. Mykhaloiko[1], R. Yatsyshyn[1], I. Dudar[2]

Thrombinuria as a link between intrarenal coagulation and inflammation in patients with glomerulonephritis

[1]Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine
[2]Bogomolets National Medical University, Kyiv, Ukraine

Citation: Mykhaloiko I, Yatsyshyn R, Dudar I. Thrombinuria as a link between intrarenal coagulation and inflammation in patients with glomerulonephritis. Ukr J Nephrol Dialys. 2025;4(88):21-30. doi: 10.31450/ukrjnd.4(88).2025.03.

Abstract. *To investigate urinary thrombin levels in patients with glomerulonephritis (GN), evaluate their relationship with the morphological type of GN, disease activity, and assess the diagnostic value of thrombinuria.*

Methods. A cross-sectional study was conducted in 72 patients with biopsy-proven GN and 40 healthy controls. Serum and urinary concentrations of thrombin, IL-6, TNF-α, and TGF-β1 were measured using ELISA. Clinical data and renal biopsy findings were analyzed. Statistical methods included correlation and group comparison tests.

Results. Urinary thrombin was markedly elevated in GN patients compared with controls (median 9.4 vs. 0.38 ng/ml; p = 0.013), while serum thrombin showed no significant difference. Thrombinuria was detected in 80.6% of patients and correlated positively with daily proteinuria (r = 0.514), urinary IL-6 (r = 0.438), and urinary TNF-α (r = 0.372). An inverse correlation was observed with urinary TGF-β1 (r = −0.534) and the chronicity index (r = −0.783), suggesting that thrombinuria characterizes active inflammatory phases, while its decline accompanies fibrotic remodeling. No significant associations were found between serum thrombin and systemic inflammatory markers.

Conclusions. Thrombinuria may reflect local activation of coagulation–inflammation pathways. It demonstrates associations with proteinuria, pro-inflammatory cytokines. These results suggest that thrombinuria could serve as a potential non-invasive biomarker of disease activity in GN; however, due to the cross-sectional design and limited sample size, the findings should be interpreted with caution and confirmed in larger longitudinal studies.

Keywords: *glomerulonephritis, thrombinuria, IL-6, TNF-α, TGF-β1, biomarkers, inflammation, coagulation.*

Article history:
Received August 26, 2025
Received in revised form October 03, 2025
Accepted October14, 2025

Conflict of interest statement. The authors declare no competing interests.

Correspondence should be addressed to Iryna Mykhaloiko: iralisn@gmail.com

УДК: 616.611-002:[612.115.12+616.151.5]

І.С. Михалойко[1], Р.І. Яцишин[1], І.О. Дудар[2]

Тромбінурія як ланка між інтраренальною коагуляцією та запаленням у хворих на гломерулонефрит

[1]Івано-Франківський національний медичний університет, м. Івано-Франківськ, Україна
[2]Національний медичний університет імені О.О. Богомольця, м.Київ, Україна

Резюме. *Метою цієї роботи було дослідити рівень тромбіну сечі пацієнтів із гломерулонефритом (ГН), оцінити його взаємозв'язок із біохімічними та морфологічними формами, активність хвороби та визначити діагностичну цінність тромбінурії.*

Методи. Проведено поперечне дослідження за участю 72 пацієнтів із морфологічно підтвердженим ГН та 40 практично здорових осіб контрольної групи. Концентрації тромбіну, IL-6, TNF-α та TGF-β_1 у сироватці крові та сечі визначали методом ІФА. Проаналізовано клінічні дані та результати біопсії нирок. Для статистичної обробки застосовували кореляційний аналіз і методи порівняння груп.

Результати. Рівень тромбіну в сечі був значно підвищений у пацієнтів із ГН порівняно з контрольною групою (медіана 9.4 проти 0.38 нг/мл; p = 0.013). Тромбінурію виявлено у 80,6% пацієнтів; вона позитивно корелювала з добовою протеїнурією (r = 0.514), рівнем IL-6 (r = 0.438) та TNF-α (r = 0.372) у сечі. Водночас встановлено зворотну кореляцію з рівнем TGF-β1 сечі (r = −0.534) та індексом хронічності (r = −0.783), що свідчить: тромбінурія відображає активність запально-коагуляційних процесів.

Висновки. Тромбінурія у хворих на ГН є наслідком локальної активації коагуляції внаслідок запалення. ЇЇ величина асоціюється з рівнями протеїнурії, прозапальних цитокінів і залежить від морфологічної форми ГН. Отримані результати свідчать, що тромбінурія може розглядатися як потенційний неінвазивний біомаркер активності гломерулонефриту; однак, з огляду на поперечний дизайн дослідження та обмежену вибірку, ці результати слід інтерпретувати з обережністю та підтвердити у більших проспективних дослідженнях.

Ключові слова: *гломерулонефрит, тромбінурія, ІЛ-6, ФНП-α, ТФР-β_1, біомаркери, запалення, коагуляція.*

Introduction. Glomerulonephritis (GN) comprises a heterogeneous group of renal disorders characterized by inflammatory injury of the glomerular apparatus. Despite the diversity of etiological triggers and morphological presentations, most forms of GN converge on common pathogenetic mechanisms. These include immune-mediated damage of the glomerular filtration barrier, activation of complement cascades, recruitment of leukocytes into the glomerular compartment, and stimulation of intraglomerular coagulation pathways [1]. The interplay of these mechanisms results in a self-perpetuating cycle, whereby inflammation amplifies coagulation, and coagulation, in turn, sustains inflammation, accelerating the progression of glomerular injury [2].

Within this context, thrombin emerges as a central player. Formed by proteolytic cleavage of prothrombin by activated factor X (Xa) in the presence of calcium, phospholipids, and cofactor V, thrombin is classically regarded as the terminal enzyme of the coagulation cascade, responsible for the conversion of fibrinogen to fibrin and clot formation. However, increasing evidence demonstrates that thrombin is not limited to hemostasis; rather, it acts as a potent signaling mediator at the interface of coagulation and inflammation [3]. By binding to and activating protease-activated receptors (PAR) expressed on endothelial cells, mesangial cells, macrophages, and platelets, thrombin induces a cascade of pro-inflammatory events. These include endothelial activation and dysfunction, increased permeability of the glomerular basement membrane, stimulation of leukocyte recruitment, and secretion of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Moreover, thrombin-mediated signaling has been implicated in the upregulation of transforming growth factor-β1 (TGF-β1), a key profibrotic cytokine that promotes extracellular matrix accumulation, mesangial expansion, and progressive glomerulosclerosis. Collectively, these processes contribute to fibrin deposition, disruption of the glomerular barrier, and advancement of both inflammatory and fibrotic injury within the kidney [4].

Physiologically, the relatively high molecular weight of thrombin (~36 kDa) prevents its filtration across an intact glomerular barrier. The detection of thrombin in urine (thrombinuria) is therefore considered an abnormal phenomenon that may reflect either

Iryna Mykhaloiko
iralisn@gmail.com

structural disruption of the glomerular filtration barrier or intraglomerular generation of thrombin with subsequent excretion into the urine [5]. This concept has been supported by several clinical investigations. Kitamoto et al. (2004) demonstrated the presence of urinary thrombin in approximately two-thirds of patients with GN, while it remained undetectable in healthy controls and in individuals with systemic disseminated intravascular coagulation [6]. A subsequent study by the same group (2015) highlighted the diagnostic utility of thrombinuria, reporting sensitivity of 70.6% and specificity of 90.1% for rapidly progressive forms of GN [7]. Furthermore, in patients with diabetic nephropathy, urinary thrombin was found with increasing frequency at advanced stages of chronic kidney disease (CKD stages III–IV), where it correlated with decreased glomerular filtration rate (GFR) and more pronounced proteinuria, thus suggesting its potential role as a marker of disease progression [8].

Nevertheless, despite accumulating evidence, the clinical role of thrombinuria remains insufficiently established. Most published studies are small in scale, cross-sectional in design, and descriptive in nature, while standardized laboratory methods for urinary thrombin detection have not been widely implemented. Several key questions remain unresolved: does urinary thrombin simply mirror structural barrier damage, or does it signify active intraglomerular coagulation-inflammation? How consistently does it correlate with traditional clinical parameters such as proteinuria, eGFR, and histological markers? Could it serve as a predictor of therapeutic response to immunosuppressive regimens or as a potential indication for anticoagulant interventions? Addressing these questions is essential for translating thrombinuria from an intriguing laboratory finding into a clinically meaningful biomarker.

In this regard, systematic evaluation of urinary thrombin in patients with GN, along with its association with clinical, biochemical, and morphological indicators of disease activity, is highly relevant. Such studies have the potential to refine our understanding of the pathophysiological crosstalk between coagulation and inflammation in glomerular disorders and may pave the way for novel diagnostic approaches and therapeutic strategies that target this critical interface.

The objective of this study was to investigate urinary thrombin levels in patients with GN and to determine their correlations with clinical, laboratory, and morphological markers of disease activity, with the aim of evaluating the diagnostic and prognostic value of thrombinuria as a biomarker of glomerular injury.

Materials and methods. In this cross-sectional observational study, we included 72 patients with primary GN admitted to the Ivano-Frankivsk Regional Clinical Hospital during 2022–2024.

This research was conducted in accordance with internationally accepted ethical standards for studies involving human subjects, including the principles of bioethics and biospecimen collection established by the World Medical Association's Declaration of Helsinki and the UNESCO Universal Declaration on Bioethics and Human Rights.

The control group consisted of 40 practically healthy individuals matched to the study cohort by key characteristics.

Of the 72 enrolled patients, the majority were male—58 individuals (80.6%; 95% CI: 69.3–88.4)—while 14 were female (19.4%; 95% CI: 11.6–30.7). The average age of the study population was 45 years, with an interquartile range of 40–49 years.

Inclusion criteria comprised age >18 years, a morphologically verified diagnosis of glomerulonephritis, and an estimated glomerular filtration rate (eGFR) above 45 ml/min/1.73 m^2. Exclusion criteria included age <18 years, refusal to participate, systemic connective tissue disorders, systemic vasculitis, diabetes mellitus (type 1 or 2), prior thromboembolic or cardiovascular events, chronic heart failure (NYHA class III–IV), acute infections of any origin, malignancies, acute or chronic hepatic failure, and psychiatric illnesses.

All 72 cases of GN included in the study were morphologically confirmed. The most frequent subtype was mesangioproliferative glomerulonephritis, diagnosed in 19 patients (26.4%; 95% CI: 17.1–37.8). Membranous nephropathy accounted for 17 cases (23.6%; 95% CI: 14.9–34.8), focal segmental glomerulosclerosis was observed in 16 patients (22.2%; 95% CI: 13.5–33.9), minimal change disease in 13 cases (18.1%; 95% CI: 10.3–28.9), and membranoproliferative (mesangiocapillary) glomerulonephritis in 7 patients (9.7%; 95% CI: 4.4–19.0).

At baseline, nephrotic syndrome was documented in 38 patients (52.8%; 95% CI: 41.2–64.2), whereas isolated urinary syndrome was observed in 34 individuals (47.2%; 95% CI: 35.8–58.8).

According to the histological subtype of GN and the clinical course, patients received pathogenetic therapy consisting of glucocorticosteroids and cytotoxic agents. All participants were prescribed either angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers. In addition, 63 patients (87.5%; 95% CI: 77.6–93.6) were treated with sodium–glucose cotransporter 2 (SGLT2) inhibitors.

The clinical diagnosis of GN was established in accordance with standard diagnostic protocols and the Kidney Disease Classification, as well as current clinical practice guidelines for the management of glomerular disorders [9]. All patients underwent a comprehensive baseline assessment that incorporated general clinical evaluation, biochemical profiling, and instrumental investigations. Biochemical analyses were performed in the certified clinical laboratory of the Ivano-Frankivsk Regional Clinical Hospital, using standardized methodologies to ensure accuracy and reproducibility of results.

Quantitative determination of thrombin concentrations in blood serum and urine was performed using enzyme-linked immunosorbent assay (ELISA) with

commercial reagent kits (MyBioSource, USA). Morning urine specimens were collected, centrifuged at 1500 rpm for 10 minutes, and 1–2 ml of the supernatant was subjected to analysis. Aliquots of supernatant were stored at –20 °C until further processing. The assay had a detection range of 0.312–20 ng/ml and an analytical sensitivity of 0.06 ng/ml.

Inflammatory markers were assessed in both serum and urine samples. The cytokines analyzed included interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and transforming growth factor-β1 (TGF-β1). IL-6 concentrations were measured using a sandwich ELISA kit (MyBioSource, USA) with a detection range of 7.8–500 pg/ml and sensitivity <2.9 pg/ml. TNF-α levels were determined by an analogous method using a kit from the same manufacturer, with a detection range of 15.6–1000 pg/ml and sensitivity <7 pg/ml. TGF-β1 was quantified using a sandwich ELISA kit (MyBioSource, USA) with a detection range of 31.25–2000 pg/ml and an analytical sensitivity of 18.75 pg/ml.

Statistical analysis was performed using STATISTICA 8 software (StatSoft, serial number STA862D175437Q). Qualitative variables were expressed as absolute numbers (n) and percentages (%) with 95% confidence intervals (CI). Quantitative data were tested for normality using the Shapiro–Wilk test. Normally distributed variables were presented as mean ± standard deviation (M±SD), while non-normally distributed data were expressed as median and interquartile range (Me (Q25–Q75)).

Comparisons between groups were carried out using Student's t-test for normally distributed variables, the Mann–Whitney U test for non-normal data, and Fisher's exact test for categorical variables. Correlations were assessed using Pearson's coefficient for normally distributed data and Spearman's coefficient for non-normally distributed data. All statistical tests were two-tailed. Given the exploratory nature of the study, no formal correction for multiple comparisons was applied. A p-value <0.05 was considered statistically significant.

Results. The levels of thrombin, pro-inflammatory cytokines, and the profibrotic marker were analyzed in both blood serum and urine of patients with GN, and the results are presented in Table 1.

Table 1

Thrombin, inflammatory, and fibrotic biomarkers in serum and urine of patients with glomerulonephritis

Parameter	Control cohort (n=40)	Patient cohort (n=72)	p-value
Thrombin in serum, ng/ml Me (Q25–Q75)	0.6 (0.4–0.8)	0.8 (0.6–1.0)	p=0.623
Thrombin in urine, ng/ml Me (Q25–Q75)	0.3 (0.3–0.4)	9.4 (6.6–14.2)	p=0.013
IL-6 in serum, pg/ml Me (Q25–Q75)	25.3 (19.6–27.5)	67.8 (33.2–93.4)	p=0.045
IL-6 in urine, pg/ml Me (Q25–Q75)	8.3 (7.1–9.5)	43.2 (28.5–77.5)	p=0.018
TNF-α in serum, pg/ml Me (Q25–Q75)	27.8 (25.3–29.7)	54.4 (33.6–70.5)	p=0.039
TNF-α in urine, pg/ml Me (Q25–Q75)	16.6 (15.1–19.3)	32.4 (23.7–43.5)	p=0.023
$TGF\beta_1$ in serum, pg/ml Me (Q25–Q75)	68.4 (51.7–75.9)	125.4 (65.2–158.5)	p=0.005
$TGF\beta_1$ in urine, pg/ml Me (Q25–Q75)	35.8 (31.5–38.4)	457.5 (325.3–656.2)	p<0.001

Abbreviations: IL-6 – interleukin-6; TNF-α – tumor necrosis factor-alpha; $TGF\text{-}\beta_1$ – transforming growth factor-beta 1; Me (Q25–Q75) – median and interquartile range.

Notes: p - statistical difference between the study group and the control group.

In this study, serum thrombin concentrations did not differ significantly between patients with glomerulonephritis and healthy controls (p = 0.623). In contrast, urinary thrombin levels were markedly elevated in the patient cohort (p = 0.013), suggesting its diagnostic relevance as a marker of glomerular injury.

Pro-inflammatory cytokine levels were consistently higher among patients. Serum IL-6 was more than doubled compared with the control group (p = 0.045), while urinary IL-6 showed an even greater increase (p = 0.018). TNF-α concentrations demonstrated a similar trend: serum levels were significantly higher in patients (p = 0.039), as were urinary levels (p = 0.023).

The most pronounced differences were observed for the profibrotic mediator TGF-β1. Serum concentrations were significantly elevated in the GN group compared to controls (p = 0.005). Urinary TGF-β1 levels showed an over tenfold increase in patients relative to healthy individuals (p < 0.001).

Collectively, these findings highlight a clear dysregulation of the coagulation–inflammation axis in glomerulonephritis, characterized by elevated thrombinuria, activation of systemic and local pro-inflammatory cytokines, and strong upregulation of fibrotic signaling through TGF-β1.

In healthy individuals, urinary thrombin concentrations did not exceed 1 ng/ml; therefore, values below this threshold were interpreted as the absence of thrombinuria. The threshold of 1 ng/ml was selected as the upper limit of urinary thrombin concentration among healthy controls and in line with previous studies [6]. Among patients with GN, thrombinuria was absent in 14 of 72 cases (19.4%; 95% CI: 10.9–29.6). Conversely, elevated urinary thrombin (>1 ng/ml) was detected in 58 patients (80.6%; 95% CI: 70.4–89.1), with a median concentration of 9.4 ng/ml (interquartile range: 6.6–14.2).

On this basis, the study cohort was stratified into two groups: Group I — patients without thrombinuria (<1 ng/ml), and Group II — patients with thrombinuria (>1 ng/ml). The principal demographic, clinical, and laboratory characteristics of both groups are summarized in Table 2.

Table 2

Baseline characteristics of patients with glomerulonephritis stratified by thrombinuria status

	Group I (n = 14)	Group II (n = 58)	p-value
Age, years Me (Q25–Q75)	43 (37–48)	46 (40–51)	0.378
Sex, male (%; 95% CI)	71.4 (44.7–88.3)	82,8 (73,1–92,4)	0.451
Sex, female (%; 95% CI)	28.6(11.7–55.3)	17,2 (7,5–26,9)	0.451
Mesangioprolifera-tive GN (%; 95% CI)	28.6 (4.7 – 52.4)	25.9 (14.5–37.2)	0.714
Membranous nephropathy (%; 95% CI)	21.4 (0 – 42.9)	24.1 (13.0–35.3)	0.569
FSGS (%; 95% CI)	21.4 (0 – 42.9)	22.4 (11.5–33.3)	0.623
Minimal change GN (%; 95% CI)	14.3 (0 – 32.7)	19.0 (8.7–29.2)	0.512
Mesangiocapillary GN (%; 95% CI)	14.3 (0 – 32.7)	8.6 (1.4–15.8)	0.615
Creatinine, μmol/L Me (Q25–Q75)	126.4 (74.5–157.6)	112.3 (7.1–141.7)	0.665
Urea, mmol/L Me (Q25–Q75)	8.7 (6.8–9.7)	8.2 (6.9–9.5)	0.856
Serum albumin, g/L Me (Q25–Q75)	32 (26–36)	24 (19–28)	0.046
eGFR, ml/min/1.73 m^2 Me (Q25–Q75)	55 (47–65)	58 (45–75)	0.654
Daily proteinuria, g/day Me (Q25–Q75)	1.8 (0.8–2.9)	4.9 (3.2–5.8)	0.001
D-dimer, mg/L Me (Q25–Q75)	0.5 (0.2–1.2)	0.8 (0.5–1.4)	0.248
Fibrinogen, g/L Me (Q25–Q75)	3.8 (2.9–4.8)	4.1 (3.9–4.9)	0.412

Abbreviations: CI – confidence interval; eGFR – estimated glomerular filtration rate; GN – glomerulonephritis; FSGS – focal segmental glomerulosclerosis; Me (Q25–Q75) – median and interquartile range.

When comparing baseline characteristics between the groups, no significant differences were observed in age, sex, morphological variants of GN, or laboratory measures, including creatinine, urea, estimated GFR, D-dimer, and fibrinogen (p > 0.05).

However, Group II demonstrated a markedly lower serum albumin level (p = 0.046) and substantially higher daily proteinuria (p = 0.001), suggesting more severe glomerular damage in patients with thrombinuria.

The correlation analysis highlighted distinct patterns linking urinary thrombin concentrations with daily proteinuria and biomarkers of inflammation and fibrosis in patients with GN.

In patients with GN, a moderate positive correlation was observed between urinary thrombin levels and daily proteinuria ($r = 0.514$; $p = 0.013$) (Fig. 1).

Significant correlations were also established between thrombinuria and pro-inflammatory cytokines, including IL-6 ($r = 0.438$; ($p = 0.021$) and TNF-α ($r = 0.372$; $p = 0.037$) (Figs. 2, 3). These findings indicate that thrombin may act not only as a marker of structural glomerular injury but also as an active mediator of inflammatory responses, enhancing cytokine production and promoting immune-inflammatory damage to renal tissue.

Of particular importance is the inverse correlation identified between urinary thrombin levels and urinary TGF-β_1 concentrations ($r = -0.534$; $p = 0.028$) (Fig. 4). Elevated TGF-β_1, a key profibrotic cytokine, was associated with reduced thrombin excretion, suggesting a pathogenetic shift from predominant inflammatory–coagulatory activity toward fibrotic remodeling processes.

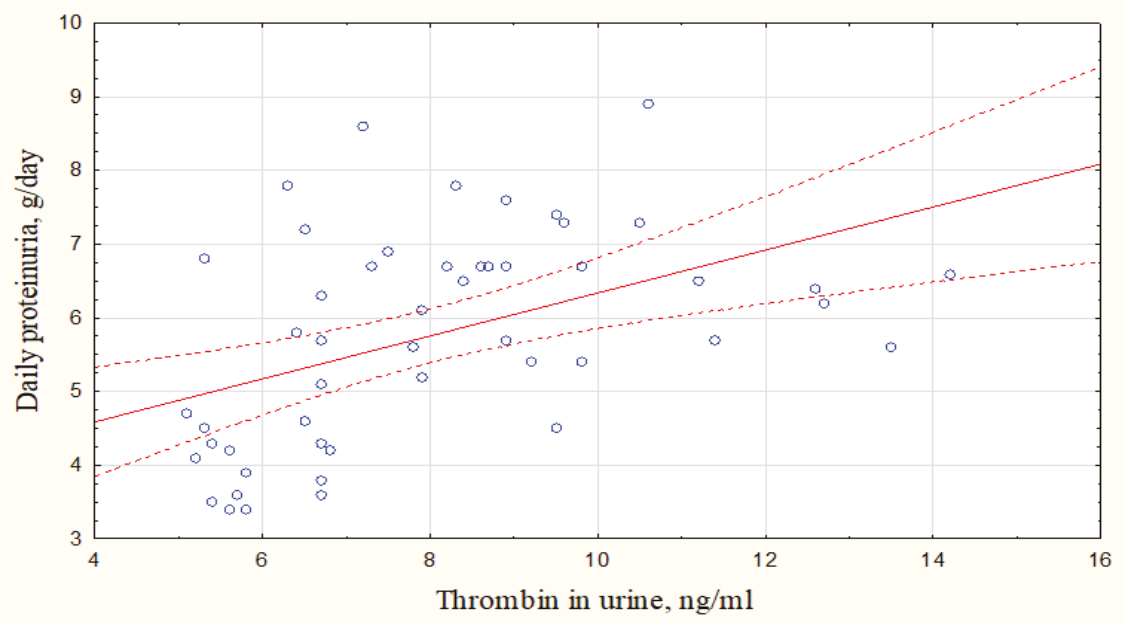

Fig. 1. Correlation between daily proteinuria (g/day) and urinary thrombin concentration (ng/ml) in patients with glomerulonephritis ($p = 0.013$).

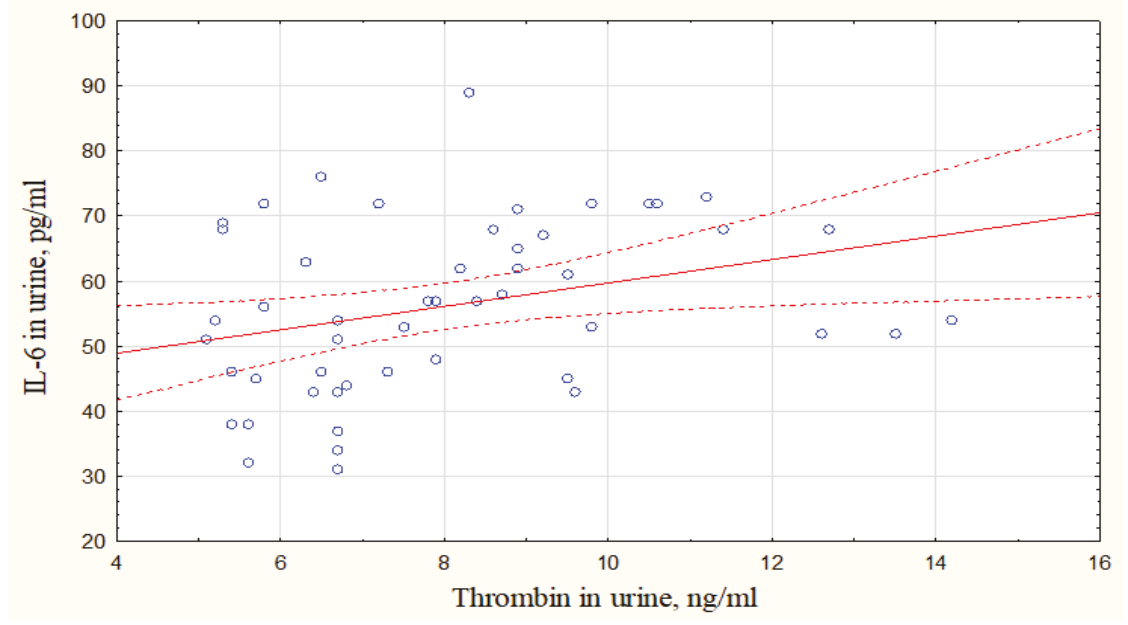

Fig. 2. Correlation between urinary interleukin-6 (pg/ml) and urinary thrombin concentration (ng/ml) in patients with glomerulonephritis ($p = 0.021$).

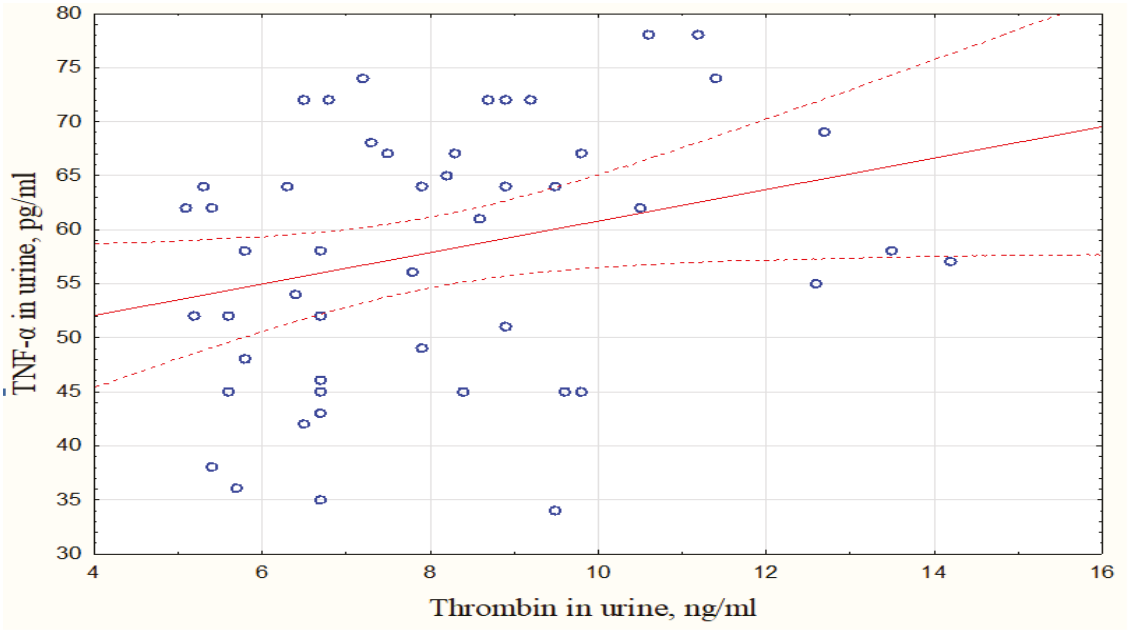

Fig. 3. Correlation between urinary tumor necrosis factor-alpha (pg/ml) and urinary thrombin concentration (ng/ml) in patients with glomerulonephritis (p = 0.037).

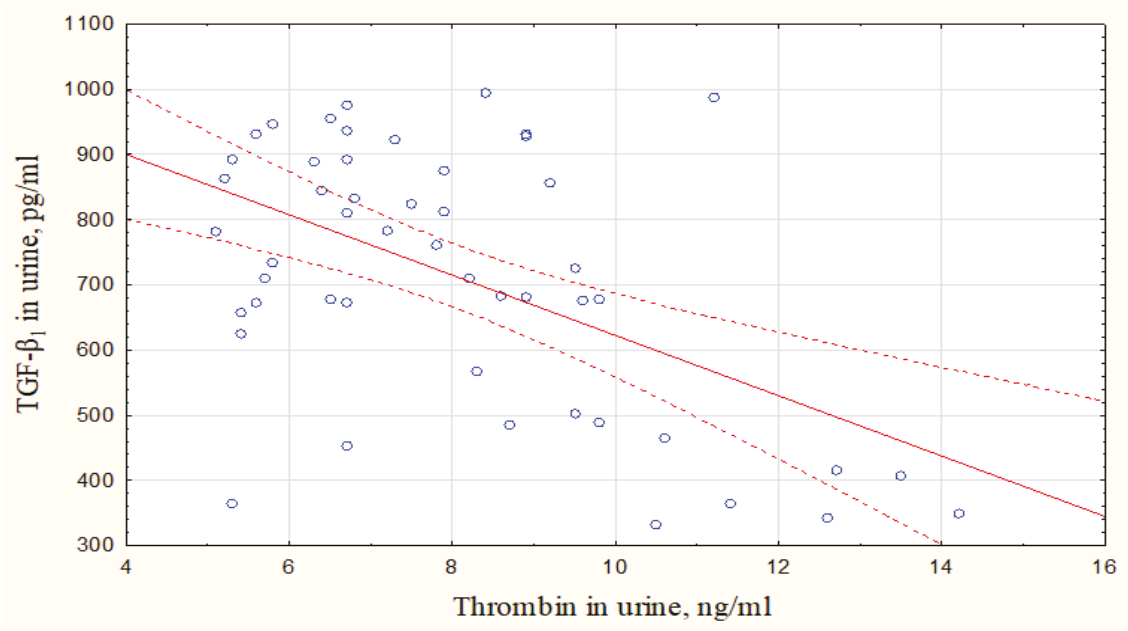

Fig. 4. Correlation between urinary transforming growth factor-beta 1 (pg/ml) and urinary thrombin concentration (ng/ml) in patients with glomerulonephritis (p = 0.028).

Additionally, the study analyzed the relationship between thrombinuria and morphological features of renal tissue injury. A strong inverse correlation was found between urinary thrombin levels and the overall chronicity score (r = –0.783; (p = 0.006), which encompassed the degree of interstitial fibrosis, tubular atrophy, and global glomerulosclerosis (Fig. 5). This indicates that lower thrombinuria levels are characteristic of advanced and chronic morphological lesions, whereas elevated thrombinuria may reflect active inflammatory–coagulatory processes in earlier disease stages.

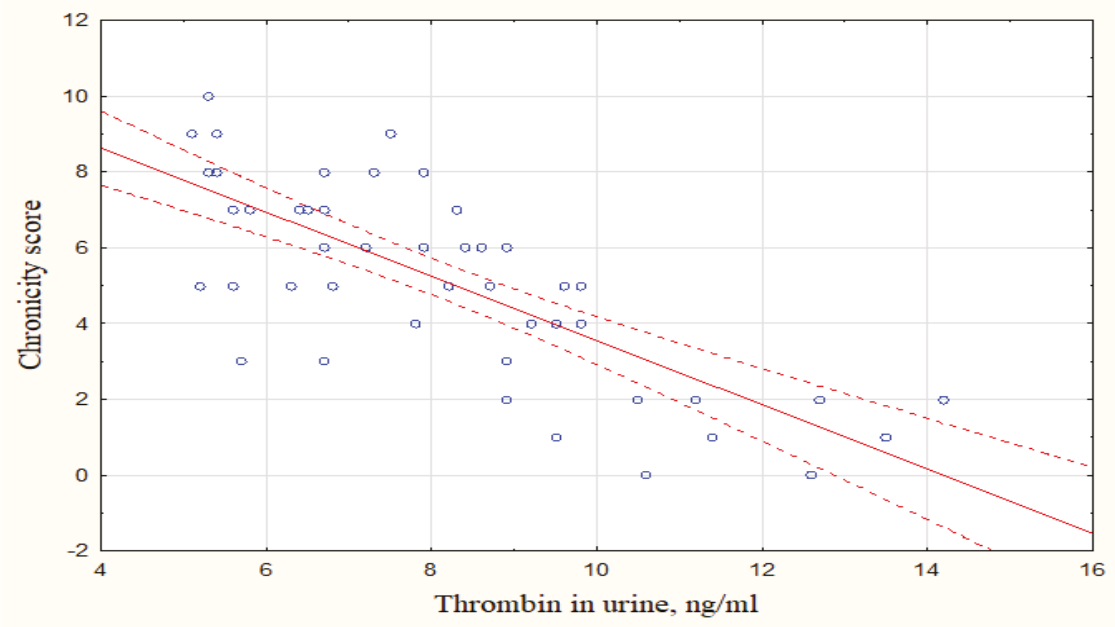

Fig. 5. Correlation between renal chronicity score and urinary thrombin concentration (ng/ml) in patients with glomerulonephritis (p = 0.006).

Taken together, these findings highlight the pathogenetic interplay between immune-inflammatory mediators, urinary thrombin generation, and structural fibrotic remodeling of the kidney. This underscores the potential of thrombinuria to serve as an integrated biomarker of both disease activity and stage in glomerular injury.

At the same time, serum analysis did not reveal statistically significant correlations between thrombin concentration and systemic inflammatory markers (IL-6, TNF-α). This result may indicate the local nature of pathological changes in renal tissue, where thrombin production and cytokine activation occur predominantly at the level of the glomeruli and tubulointerstitium, rather than being reflected in the systemic circulation.

The obtained data emphasize the importance of assessing urinary markers for studying the pathogenesis of GN, since they reflect the local activity of inflammatory and coagulation cascades, whereas plasma analysis may underestimate these changes.

Discussion. In the present study, we demonstrated a significant increase in urinary thrombin levels in patients with GN, while serum thrombin concentrations did not differ significantly from those of the control cohort. Thrombinuria was detected in over 80% of patients, underscoring its potential role as a local marker of glomerular injury. These findings align with earlier reports by Kitamoto et al. (2004, 2015), who highlighted the diagnostic value of thrombinuria in rapidly progressive forms of GN, with high sensitivity and specificity [6, 7]. Our findings are consistent with more recent evidence in diabetic nephropathy, where the presence of thrombin in urine was associated with both proteinuria and a decline in GFR, indicating that this biomarker may have wider relevance across various renal diseases [8].

The correlations identified in our study further strengthen the pathogenetic link between thrombin generation and disease activity. Urinary thrombin levels were positively associated with daily proteinuria and urinary concentrations of IL-6 and TNF-α, two key pro-inflammatory cytokines. This observation is in line with experimental studies demonstrating that thrombin, through activation of protease-activated receptors, induces cytokine secretion and endothelial dysfunction, thereby sustaining renal inflammation [3]. Comparable associations were described in lupus nephritis, where thrombin activity was linked to intrarenal inflammatory burden and histological severity [10]. Such cross-disease consistency supports the hypothesis that thrombinuria reflects not merely passive leakage but active local intraglomerular coagulation and immune activation.

A particularly noteworthy observation, consistent with both our findings and prior reports, is that free thrombin in the urine may be generated locally within the glomeruli under the influence of tissue factor released by mesangial cells in response to pro-inflammatory cytokines. This supports the concept that thrombinuria should be regarded not only as an indicator of glomerular barrier disruption but also as a manifestation of a localized, DIC-like process within the kidneys [11, 12]. The absence of thrombinuria in patients with systemic disseminated intravascular coagulation further underscores this local nature [13]. Importantly, analogous phenomena have been described beyond nephrology: thrombin has been identified in bronchoalveolar lavage fluid of patients with pulmonary fibrosis, as well as immunohistochemically localized in brain tissue of individuals with Alzheimer's disease [14]. Together, these findings highlight thrombin's universal role as a marker of tissue-specific inflammatory–coagulation activity across multiple organ systems.

Interestingly, our study also revealed an inverse relationship between urinary thrombin and urinary TGF-β1 levels, as well as between thrombinuria and the chronicity index of renal pathology. The inverse association between urinary thrombin and TGF-β1/chronicity index may indicate a transition from active inflammatory-coagulative processes to fibrotic remodeling. In early disease stages, thrombin generation reflects active glomerular injury, while in advanced fibrosis, its reduction parallels loss of cellular activity and progressive matrix accumulation. These findings may indicate a pathogenetic shift: elevated thrombinuria appears to characterize earlier and more inflammatory phases of GN, whereas reduced urinary thrombin accompanies advanced fibrosis and irreversible structural remodeling. This interpretation resonates with observations in chronic kidney disease of various etiologies, where urinary thrombin levels were shown to decline in late-stage fibrosis (Kitamoto et al., 2021) [8]. Taken together, these results position thrombinuria as a dynamic biomarker reflecting both activity and chronicity of glomerular injury.

The absence of correlations between serum thrombin and systemic markers of inflammation (IL-6, TNF-α) further underscores the local nature of these processes. This is consistent with previous reports demonstrating that plasma-based assays may underestimate intrarenal activity, whereas urinary biomarkers provide a more sensitive reflection of localized pathophysiological changes [15]. The parallels with other organ systems where thrombin accumulates locally—without systemic elevation—reinforce its significance as a marker of localized coagulation–inflammation interplay.

From a clinical perspective, our findings suggest that urinary thrombin could be integrated into diagnostic algorithms as a non-invasive marker of disease activity in GN. Elevated thrombinuria may help identify patients at higher risk of progressive injury, guide therapeutic decision-making, and potentially serve as a surrogate marker for treatment response. Moreover, considering the established profibrotic and pro-inflammatory role of thrombin, targeting thrombin-mediated pathways may open new therapeutic avenues.

Other urinary biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) and monocyte chemoattractant protein-1 (MCP-1) have also been extensively investigated as indicators of renal inflammation and tubular injury. NGAL reflects early tubular stress and neutrophil activation, while MCP-1 is a chemokine responsible for monocyte recruitment and interstitial inflammation [16, 17]. In this context, thrombinuria complements these markers by emphasizing the coagulation–inflammation axis that characterizes glomerular injury. However, thrombinuria is not specific to glomerulonephritis alone—it has also been observed in diabetic nephropathy and lupus nephritis [7, 8]. Therefore, its diagnostic and prognostic significance should be interpreted in conjunction with clinical findings and histopathological evaluation.

Limitations. The single-center, cross-sectional design and relatively small cohort limit the generalizability of the findings. Furthermore, the absence of longitudinal follow-up precluded assessment of thrombinuria dynamics under treatment or its predictive value for renal outcomes. Due to the cross-sectional design, causal relationships between thrombinuria and inflammatory or fibrotic parameters cannot be established. Despite these limitations, our results provide compelling evidence that thrombinuria is a promising biomarker of local renal inflammation and coagulation, warranting further validation in larger, multicenter, prospective studies.

Conclusions:

1. Thrombinuria was observed in a substantial proportion of patients with glomerulonephritis.

2. Urinary thrombin showed positive associations with proteinuria and local inflammatory cytokines (IL-6, TNF-α), suggesting a possible link between coagulation activity and intrarenal inflammation.

3. Inverse correlations between urinary thrombin, TGF-β_1 levels, and the chronicity index may reflect a shift from inflammatory to fibrotic processes as glomerular injury advances.

4. Thrombinuria may represent a promising non-invasive biomarker of renal inflammatory activity; however, given the cross-sectional design and limited cohort size, these findings should be interpreted with caution. Further longitudinal and mechanistic studies are needed to confirm these preliminary observations.

Ethics statement. The study protocol was reviewed and approved by the Ethics Committee of Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine (Protocol No. 124/21, November 29, 2021). All participants provided written informed consent prior to enrollment in the study and for the collection and analysis of their clinical and laboratory data.

Conflict of interest. The authors declared no competing interests.

Findings. The research was performed within the framework of the institutional project "Immunological and biochemical disorders in internal organ pathology: differentiated approaches to treatment" (State registration No. 0124U002921).

Funding source. The authors received no financial support for the research.

Information about the contribution of the authors.

I. Mykhaloiko: literature search, study design planning, data analysis, manuscript writing and submission;

R. Yatsyshyn: concept and management of the work;

I. Dudar: concept and management of the paper.

References:

1. *Dudar I, Mykhaloiko I.* The role of hemostatic disorders in the progression of chronic kidney disease. Ukr J Nephr Dial. 2019;4(64):49-55. doi: 10.31450/ukrjnd.4(64).2019.07.
2. *Esmon CT.* Crosstalk between inflammation and thrombosis. Maturitas. 2004;47(4):305–314. doi: 10.1016/j.maturitas.2003.10.015.
3. *Coughlin SR.* Thrombin signalling and protease-activated receptors. Nature. 2000;407(6801):258–264. doi: 10.1038/35025229.
4. *Cunningham MA, Rondeau E, Chen X, Coughlin SR, Holdsworth SR, Tipping PG.* Protease-activated receptor 1 mediates thrombin-dependent, cell-mediated renal inflammation in crescentic glomerulonephritis. J Exp Med. 2000;191(3):455–462. doi: 10.1084/jem.191.3.455.
5. *Qiu Z, Pang X, Xiang Q, Cui Y.* The Crosstalk between Nephropathy and Coagulation Disorder: Pathogenesis, Treatment, and Dilemmas. J Am Soc Nephrol.2023;34(11):1793-1811. doi: 10.1681/asn.0000000000000199.
6. *Kitamoto Y, Tomita K, Imamura T.* Assessment of thrombin in the urine of glomerulonephritic patients by enzyme-linked immunosorbent assay. Ann Clin Biochem. 2004;41(2):133–137. doi: 10.1258/000456304322880023.
7. *Kitamoto Y, Arizono K, Fukui H, Tomita K, Kitamura H, Taguma Y, Imamura T.* Urinary thrombin: a novel marker of glomerular inflammation for the diagnosis of crescentic glomerulonephritis. PLoS One. 2015;10(3):e0118704. doi: 10.1371/journal.pone.0118704.
8. *Kitamoto Y, Imamura T, Taguma Y, Tomita K.* Urinary Thrombin as a Marker of Glomerular Inflammation Associated with Renal Injury in Type 2 Diabetes. Tohoku J Exp Med. 2021;253(1):41-49. doi: 10.1620/tjem.253.41.
9. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group. KDIGO 2021 clinical practice guideline for the management of glomerular diseases. Kidney Int. 2021;100:1-276. doi: 10.1016/j.kint.2021.05.021.
10. *de Zubiria Salgado A, Herrera-Diaz C.* Lupus nephritis: an overview of recent findings. Autoimmune Dis. 2012;2012(1):849684. doi: 10.1155/2012/849684.
11. *Lin KY, Kwong GA, Warren AD, Wood DK, Bhatia SN.* Nanoparticles that sense thrombin activity as synthetic urinary biomarkers of thrombosis. ACS Nano. 2013;7(10):9001-9. doi: 10.1021/nn403550c.
12. *Mykhaloiko I.* Urinary thrombin as a marker of local disseminated intravascular coagulation in patients with chronic kidney disease. Med. perspekt. 2021;26(4):81-6. doi: 10.26641/2307-0404.2021.4.248157.
13. *Levi M, Ten Cate H.* Disseminated intravascular coagulation. N Engl J Med. 1999;341(8):586–592. doi: 10.1056/NEJM199908193410807.
14. *Howell DC, Goldsack NR, Marshall RP, McAnulty RJ, Starke R, Purdy G, et al.* Direct thrombin inhibition reduces lung collagen accumulation, matrix metalloproteinase-9 expression, and neutrophil recruitment in pulmonary fibrosis. Am J Pathol. 2001;159(4):1383-1395. doi: 10.1016/s0002-9440(10)62525-4.
15. *Xiao M, Tang D, Luan S, Hu B, Gong W, Pommer W, et al.* Dysregulated coagulation system links to inflammation in diabetic kidney disease. Front Clin Diabetes Healthc. 2023;4:1270028. doi: 10.3389/fcdhc.2023.1270028.
16. *Zhang WR, Parikh CR.* Biomarkers of Acute and Chronic Kidney Disease. Annu Rev Physiol. 2019;81:309-333. doi: 10.1146/annurev-physiol-020518-114605.
17. *Bolignano D, Donato V, Coppolino G, Campo S, Buemi A, Lacquaniti A, Buemi M.* Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. Am J Kidney Dis. 2008;52(3):595-605. doi: 10.1053/j.ajkd.2008.01.020.