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Pre-existing serum indoxyl sulfate and COVID-19 outcomes in patients undergoing hemodialysis: A retrospective cohort study

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Abstract. Patients undergoing hemodialysis (HD) are at increased risk of severe complications from COVID-19 due to compromised immune function and comorbidities. This retrospective study aimed to investigate the association between pre-existing serum indoxyl sulfate (IS) concentrations and COVID-19 outcomes in HD patients.

Methods. Data on pre-existing IS and proinflammatory cytokines, such as interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF- α) were extracted from an existing patient database. The patients were followed up for 1.5 years and compared according to median serum IS concentration: low-IS (< 22.2 μ g/mL) and high-IS (\geq 22.2 μ g/mL) groups. The primary outcomes focused on assessing the risk and severity of COVID-19 infection.

Results. A total of 56 patients aged 62 (56-67) years with a dialysis vintage of 37.5 (30-168) months were included in the analysis. Serum levels of IS were significantly correlated with Kt/V values ($p = 0.043$), arterial hypertension ($p = 0.001$), IL-6 ($p = 0.023$), MCP-1 ($p = 0.023$), and TNF- α ($p = 0.033$) concentrations. Elevated serum IS levels were significantly associated with an increased risk of COVID-19 infection ($p < 0.0001$) and a higher likelihood of hospitalization ($p = 0.03$). Patients with higher IS levels exhibited more severe lung involvement ($p < 0.0001$) and a greater need for respiratory support ($p = 0.004$). A serum IS concentration of 21.5 μ g/mL was the optimal threshold for predicting COVID-19 infection in HD patients (sensitivity of 83.4% and specificity of 92.3%, $p < 0.0001$).

Conclusion: Our study highlights the detrimental impact of serum IS on COVID-19 infection and its clinical outcomes in patients undergoing HD. Further research is warranted to elucidate the underlying mechanisms and explore potential therapeutic strategies targeting IS in this population.

Keywords: hemodialysis, COVID-19, indoxyl sulfate, risk, hospitalization, cytokines.

Conflict of interest. The authors declare no conflict of interest.

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Передінфекційна концентрація індоксил сульфату та наслідки COVID-19 у пацієнтів, які лікуються методом гемодіалізу: ретроспективне когортне дослідження

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Резюме. Пацієнти, які лікуються методом гемодіалізу (ГД) належать до групи високого ризику важких ускладнень COVID-19. Це ретроспективне дослідження мало на меті вивчити взаємозв'язок між передінфекційною концентрацією індоксилу сульфату (ІС) сироватки та клінічними наслідками COVID-19 у ГД пацієнтів.

Методи. Дані щодо передінфекційної концентрації ІС та прозапальних цитокінів, таких як інтерлейкін-6 (ІЛ-6), моноцитарний хемоатрактантний протеїн-1 (МХП-1) та фактор некрозу пухлин альфа (ФНП- α), були вилучені з наявної бази даних пацієнтів. Період спостереження склав 1,5 роки; аналіз проводили відповідно до медіанних значень концентрації ІС (< 22,2 мкг/мл та \geq 22,2 мкг/мл). Основні результати спрямовувались на оцінку ризику та важкості інфікування COVID-19.

Результати. До аналізу включено 56 ГД пацієнтів у віці 62 (56-67) років з тривалістю діалітичного лікування 37,5 (30-168) місяців. Концентрація ІС в сироватці мала статистично значущий кореляційний зв'язок з Кт/V ($p = 0,043$), артеріальною гіпертензією ($p = 0,001$), ІЛ-6 ($p = 0,023$), МХП-1 ($p = 0,023$), та ФНП- α ($p = 0,033$). Підвищені рівні ІС (\geq 22,2 мкг/мл) були асоційовані зі збільшеним ризиком інфікування COVID-19 ($p < 0,0001$) та імовірністю госпіталізації ($p = 0,03$). Висока концентрація ІС асоціювалась з важким ураженням легень ($p < 0,0001$) та потребою у кисневій підтримці ($p = 0,004$). Концентрація ІС сироватки 21,5 мкг/мл може бути предиктором інфікування COVID-19 у ГД пацієнтів (чутливість 83,4% та специфічність 92,3%, $p < 0,0001$).

Висновки. Наше дослідження підкреслює негативний вплив ІС сироватки на ризик інфікування та клінічні наслідки COVID-19. Подальші дослідження необхідні для визначення основних механізмів та потенційних терапевтичних стратегій, спрямованих на ІС у цій популяції хворих.

Ключові слова: гемодіаліз, COVID-19, індоксил сульфат, ризик, госпіталізація, цитокіни.

Introduction. The COVID-19 pandemic has caused significant morbidity and mortality worldwide, with certain populations experiencing higher susceptibility and worse outcomes [1, 2]. Patients with pre-existing chronic conditions, such as chronic kidney disease (CKD) requiring hemodialysis (HD), have been identified as particularly vulnerable to severe COVID-19 infection and its complications [2, 3].

Chronic inflammation is a systemic inflammatory state that arises from persistent immune system activation, often triggered by long-term infections, autoimmune diseases, or metabolic dysregulation [4]. In patients with CKD, chronic inflammation is a prevalent and well-documented condition driven by factors such as the accumulation of uremic toxins, altered gut microbiota, oxidative stress, and dysregulated immune responses [5–7]. These inflammatory processes have been associated with accelerated atherosclerosis and increased cardiovascular and all-cause mortality risk in patients undergoing HD [7–9].

Indoxyl sulfate (IS), a uremic toxin generated by gut microbiota from dietary tryptophan, has emerged

as a potential key mediator of chronic inflammation in CKD [10]. It has been shown to activate inflammatory pathways and promote oxidative stress, contributing to the systemic inflammation observed in patients treated with maintenance HD [10–13]. Elevated levels of IS have been associated with various adverse outcomes in CKD, including cardiovascular disease and mortality [14, 15]. However, the interplay between IS concentrations and COVID-19 outcomes in patients undergoing HD has never been studied.

We hypothesized that pre-existing high concentrations of IS may promote inflammatory response and influence the severity of COVID-19 infection and its associated outcomes in this patient population. Understanding the impact of this uremic toxin on COVID-19 outcomes in patients undergoing HD could provide valuable insights into risk stratification, clinical management, and potential interventions.

Therefore, the present study aimed to retrospectively explore the relationship between pre-existing serum concentrations of IS and proinflammatory markers and COVID-19 outcomes in patients undergoing HD.

Patients and Methods. This study was conducted as part of an ongoing project titled “Mechanisms of Development and Therapeutic Targets of Post-COVID Syndrome in Dialysis Patients” (National Study Registration Number 0122U000144) at the State Institution “Institute of Nephrology of the National Academy of Medical Science of Ukraine” in Kyiv, Ukraine. The

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study adhered to the principles outlined in the Declaration of Helsinki and took place between November 2021 and May 2022. The study protocol (protocol number: 2-2021, dated April 6, 2021) was approved by the Institute's Ethics Committee.

Study cohort and outcomes. The research employed a retrospective design utilizing an existing patient database initially established for a separate scientific project. From this database, relevant data on IS and cytokines were extracted and analyzed exclusively for this study.

Inclusion criteria for participants encompassed patients undergoing maintenance HD for at least 3 months before enrollment, the availability of data on IS and cytokine levels measured at the same time point prior to the COVID-19 pandemic in the patient's database, and a clinically stable condition with an adequate-

ly functioning arteriovenous fistula and Kt/V (dialysis adequacy) ≥ 1.2 . Additionally, patients infected with COVID-19 needed to have a documented diagnosis of the disease.

Exclusion criteria involved patients with missing or incomplete data, patients who underwent kidney transplantation or transitioned from peritoneal dialysis to HD, and patients with significant conditions that may confound the analysis of COVID-19 outcomes (such as diabetes mellitus, a history of cardiovascular events, immunosuppressive treatment, systemic or malignant diseases, or acute inflammatory processes).

The primary outcomes of interest centered on assessing the severity of COVID-19 infection and examining the incidence of hospitalizations and mortality associated with the disease (Fig. 1).

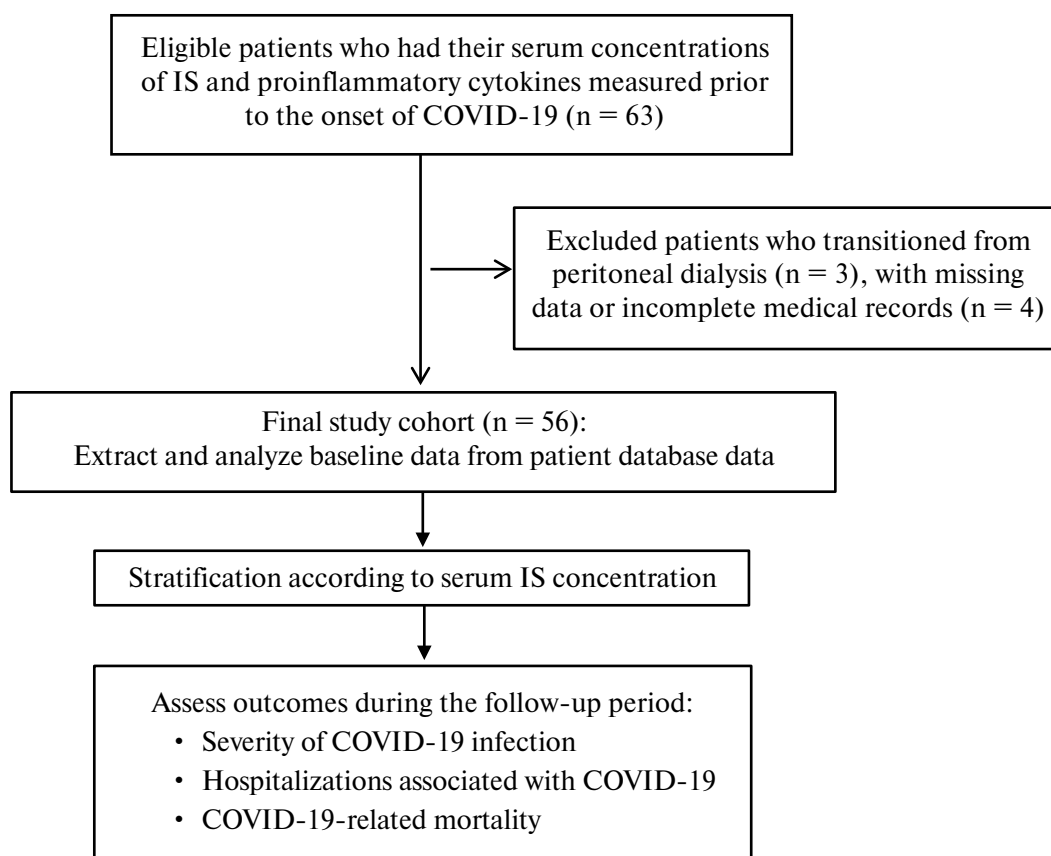


Fig. 1. Flow chart diagram of the study design.

The follow-up period was 1.5 years (18 months) after the initial diagnosis of COVID-19. The study endpoint was defined as reaching the end of the follow-up period, experiencing mortality or encountering a loss to a follow-up event. The data collection concluded on December 31, 2021, serving as the designated cutoff date for the study.

Data collection. The data collection process involved retrieving specific variables of interest, including patient demographics, laboratory results, and COVID-19 experience and outcomes. These variables included sex, age, duration of HD treatment, body

mass index (BMI), comorbidities, and measurements of specific biomarkers, such as IS, interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF- α), C-reactive protein (CRP), hemoglobin (Hb), cholesterol, electrolytes, parathyroid hormone (PTH), and albumin.

All biochemical parameters were determined using the "Flexor Junior" automatic analyzer (Netherlands), while hematological blood parameters were examined using the "ABX Micros-60" analyzer (France).

The concentration of IS was determined using Obermeyer's spectrophotometric method. The cyto-

kine assay was performed using the SunRise Touch-Screen immunoassay analyzer and commercially available ELISA kits (IBL International GmbH, Hamburg, Germany).

The prevalence of lung involvement in patients with COVID-19-associated pneumonia was assessed based on chest computer tomography (CT) data using a widely accepted grading system: Grade 1 indicates lung involvement less than 5%, Grade 2 indicates lung involvement between 5-25%, Grade 3 indicates lung involvement between 26-49%, Grade 4 indicates lung involvement between 50-75%, and Grade 5 indicates lung involvement over 75% of lung tissue.

Statistical analysis. The statistical analysis and graphical representations were performed using MedCalc Statistical Software version 20.2.18 (MedCalc Software Ltd., Ostend, Belgium). The normality of the data was assessed using the Kolmogorov–Smirnov test (dK–S). As the data did not follow a normal distribution, descriptive analysis was presented using the median (Me) and interquartile range (Q25–Q75),

while comparative analysis utilized the non-parametric Mann–Whitney U test.

Correlation analysis was conducted using Spearman’s method to assess the relationships between variables. Differences in frequencies between groups were evaluated using the chi-square test (χ^2).

To determine the optimal cut-off point for serum IS concentration in predicting COVID-19 infection, receiver operating characteristic (ROC) analysis was employed. Kaplan-Meier analysis was performed to assess the risk of hospitalization, and the log-rank test was used to compare the survival curves between the groups.

Results. Out of the 56 patients included in the study, 31 (55.4%) were male and 25 (44.6%) were female. The average age of the patients was 62 (56-67) years, and their duration of HD treatment was 37.5 (30-168) months. At baseline, the serum concentrations of IS ranged from 2 to 68.2 $\mu\text{g}/\text{mL}$, with a median value of 22.2 (16.7- 48) $\mu\text{g}/\text{mL}$. For further analysis, the patients were categorized into two groups: low-IS (< 22.2 $\mu\text{g}/\text{mL}$) and high-IS ($\geq 22.2 \mu\text{g}/\text{mL}$) (Table 1).

Table 1

Baseline characteristics of the patients stratified by median serum IS value

	Low-IS Group (n = 21)	High-IS Group (n = 35)	p-value
Demographic and routine clinical data			
Male sex, n (%)	12 (57.1%)	19 (50%)	0.61
Age, years	66 (60-67)	62 (56-65)	0.29
Dialysis vintage, years	88 (29-126)	33 (30-84)	0.46
Diabetes mellitus	4 (19%)	12 (34.3%)	0.22
BMI, kg/m ²	26 (23-30.5)	26 (23.6-27.6)	0.74
Kt/V	1.4 (1.3-1.45)	1.2 (1.2-1.32)	0.001
Systolic blood pressure, mm Hg	120 (110-120)	130 (130-150)	0.008
Diastolic blood pressure, mm Hg	80 (70-85)	80 (70-100)	0.66
Hb, g/L	106 (92.2-113)	98.4 (90.1-109)	0.02
Serum albumin, g/L	38.5 (36.1-41.6)	36.9 (35.5-41.7)	0.62
Calcium, mmol/L	2.3 (2.25-2.43)	2.05 (1.98-2.2)	0.02
Phosphorus, mmol/L	1.29 (1.19-1.47)	2.1 (1.7-2.5)	0.003
iPTH, ng/L	123 (110-283)	358 (77.4-639)	0.59
Total cholesterol, mmol/L	3.2 (3.08-3.9)	4.4 (4.3-4.6)	0.003
Inflammatory markers			
IS, $\mu\text{g}/\text{mL}$	17 (15.9-18.2)	26 (22.2-50)	0.0002
CRP, mg/L	7.4 (5.6-11.3)	11.7 (6.9-14.8)	0.0001
IL-6, pg/mL	0.9 (0.5-3.2)	22.7 (9.8-25.9)	0.0003
MCP-1, pg/mL	266 (227.4-300)	389 (305-401)	0.008
TNF- α , pg/mL	0.6 (0.35-3.6)	2.25 (0.7-4.3)	0.04

Abbreviation: BMI – body mass index, CRP – C-reactive protein, Hb – hemoglobin, IL-6 – interleukin-6, iPTH – intact parathyroid hormone, MCP-1 – monocyte chemoattractant protein-1, TNF- α – tumor necrosis factor-alpha.

As shown in Table 1, the high-IS group of patients exhibited lower values of Kt/V, Hb, and calcium, as well as higher levels of systolic blood pressure, phosphate, PTH, and cholesterol when compared to the low-IS group. Additionally, the high-IS group displayed ele-

vated levels of all inflammatory markers in comparison to the low-IS group. Serum levels of IS were significantly correlated with Kt/V values ($r = 0.36$, $p = 0.043$), arterial hypertension ($r = 0.72$, $p = 0.001$), IL-6 ($r = 0.35$, $p = 0.023$), MCP-1 ($r = 0.35$, $p = 0.023$), TNF- α

($r = 0.37, p = 0.033$), and CRP ($r = 0.69, p = 0.005$) concentrations.

During the follow-up period of 18 months, 35 (62.5%) individuals had experienced COVID-19, 14 (40%) of them required hospitalization, and 5 (14.3%) died. The prevalence of COVID-19 infection and its associated hospitalization was significantly higher in the high-IS group compared to the low-IS group: 31 (88.6%) vs. 4 (19%), $\chi^2 = 23.2, p < 0.0001$ and 12 (34.3%) vs. 2 (9.5%), $\chi^2 = 4.6, p = 0.003$, respectively.

Further analysis of the COVID-19-infected group revealed a statistically significant higher concentration of

IS in the serum of patients who required oxygen support compared to non-hospitalized patients: 36.8 (29-54.3) vs. 27.5 (23.1-48.7) $\mu\text{g/mL}$, $p = 0.004$. Moreover, the serum IS concentration showed a strong positive correlation with the extent of COVID-19-associated pulmonary involvement in patients undergoing HD (Fig. 2).

The ROC curve analysis determined that a serum IS concentration of 21.5 $\mu\text{g/mL}$ was the optimal threshold for predicting COVID-19 infection in HD patients, with a sensitivity of 83.4% and a specificity of 92.3%. The area under the ROC curve was 0.92 (95% CI 0.85; 0.99), $p < 0.0001$ (Fig. 3).

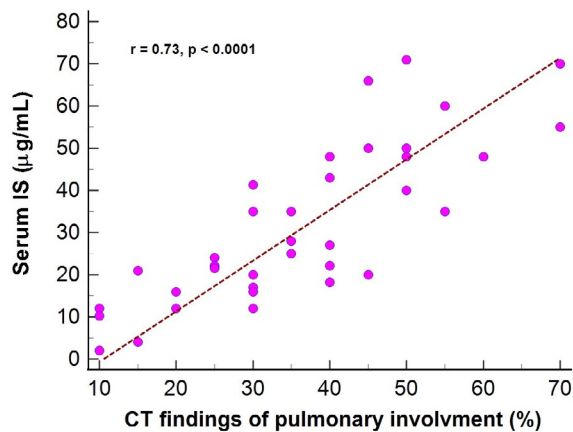


Fig. 2. Correlation between the concentration of serum IS and the extent of COVID-19-associated lung involvement in patients undergoing HD.

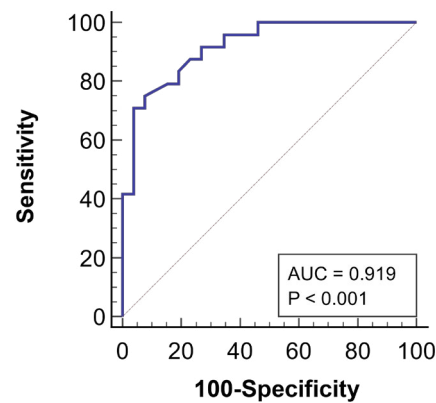
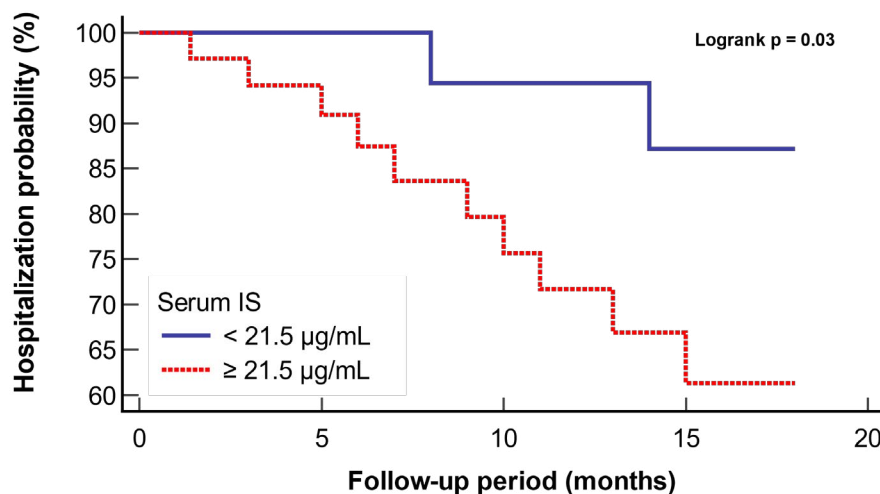


Fig. 3. ROC curve depicting the threshold value of serum IS for predicting COVID-19 infection in HD patients.

Furthermore, Kaplan-Meier analysis stratified by serum IS levels demonstrated a significant increase in

the risk of hospitalization among patients with a serum IS concentration $\geq 21.5 \mu\text{g/mL}$ (Fig. 4).



Number at risk

Serum IS: < 21.5 $\mu\text{g/mL}$

21	18	13	8	0
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Serum IS: $\geq 21.5 \mu\text{g/mL}$

35	26	19	10	0
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Fig. 4. Hospitalization probability of COVID-19-infected patients undergoing HD stratified by serum IS concentration (Kaplan–Meier analysis).

Although all the deceased patients were from the high-IS group, the sample size was too small to establish a statistically significant difference in mortality between the groups ($\chi^2 = 3.4$, $p = 0.06$).

Discussion. Pre-existing conditions, such as CKD, diabetes, obesity, cardiovascular diseases, and rheumatic diseases, have consistently been identified as significant risk factors for more severe outcomes and higher mortality rates in individuals who contract COVID-19 [3, 16–19]. Chronic inflammation underlying these conditions may partially explain the poor outcomes of COVID-19 in these vulnerable populations [20]. Despite the significantly higher risk of COVID-19-associated mortality (4.5-fold) observed in the HD population compared to other patient groups [21] existing studies exploring the relationship between chronic inflammation and COVID-19 outcomes in this specific cohort of patients are scarce and yield conflicting results. On the one hand, chronic inflammation, characterized by prolonged immune system activation, can be further aggravated by the onset of HD [22]. This altered immune response in individuals infected with COVID-19 can lead to the development of severe complications [20]. On the other hand, it has been proposed that the persistent activation of the immune system seen in chronic inflammation could potentially confer some level of protection against COVID-19 in patients undergoing HD [23]. In this context, the present study was conducted to investigate the potential influence of pre-existing uremic milieu, as indicated by serum IS concentration and the subsequent proinflammatory cytokine response, on the severity of COVID-19 infection and its associated adverse outcomes in patients undergoing HD.

Consistent with previous experimental [11, 24] and clinical [12, 25] findings, our study demonstrated a direct correlation between serum IS levels and the concentrations of proinflammatory markers, such as CRP, IL-6, MCP-1, and TNF- α . These findings provide further evidence of the proinflammatory effects of IS in patients undergoing HD. Moreover, patients with higher serum IS concentrations had a significantly increased risk of COVID-19 infection and its associated hospitalization. These observations can potentially be explained by several factors. First, elevated serum IS levels have been associated with altered humoral response that affects the body's ability to mount an effective defense against COVID-19 [26]. Second, IS has been implicated in endothelial dysfunction, which is characterized by impaired function of the blood vessel lining [12]. Endothelial dysfunction can lead to increased vascular permeability, inflammation, and clotting, all of which are involved in the pathogenesis of COVID-19 [12, 27]. Elevated serum IS levels may contribute to endothelial dysfunction, thereby increasing the risk of COVID-19 and its complications. Third, IS has been shown to modulate the renin–angiotensin–aldosterone system (RAAS) [28, 29]. Dysregulation of the RAAS has been implicated in the severity of COVID-19 [30]. Given this association, elevated IS levels could potentially contribute to the dysregulation of the RAAS and

thereby increase the risk and severity of COVID-19 in patients undergoing HD.

Furthermore, we demonstrated that the accumulation of IS in the serum can contribute to the development of severe pulmonary involvement, as assessed by chest CT grading. The presence of elevated IS levels may contribute to the systemic inflammatory state observed in patients undergoing HD, leading to heightened susceptibility to severe lung injury upon COVID-19 infection. Our findings align with previous research indicating that IS plays a toxicophysiological role as a mediator involved in the kidney–lung axis [31]. It is associated with oxidative stress, inflammation, endothelial dysfunction, and hemostatic disorders, which are all key factors in the pathogenesis of COVID-19 pneumonia [12, 13, 27, 32]. Importantly, both CKD and COVID-19-associated pneumonia can impact pulmonary tissue and vasculature [33–35], further supporting the relevance of our findings.

It is important to acknowledge that our study has several limitations that should be taken into consideration. The retrospective design and reliance on existing patient data could introduce biases and limitations inherent in such an approach. Additionally, the relatively small sample size and single-center nature of the study limit the generalizability of our findings. However, despite these limitations, our study holds significance, as it is the first to demonstrate the detrimental effect of elevated IS levels on the risk and severity of COVID-19. This underscores the importance of addressing chronic inflammation and, consequently, dialysis adequacy in the management of patients undergoing HD with COVID-19. Future studies with larger sample sizes and multicenter designs are warranted to further validate and expand upon our findings.

Conclusions. Our study provides preliminary evidence of a potential association between pre-existing chronic inflammation, represented by elevated serum IS levels, and the risk of COVID-19 infection and its outcomes in patients undergoing HD. Serum IS concentration ≥ 21.5 $\mu\text{g/mL}$ can be served as a predictive marker for COVID-19 infection risk and severity. Future studies should focus on elucidating the underlying mechanisms linking chronic inflammation, COVID-19 pathogenesis, and patient outcomes to guide the development of targeted interventions and improve clinical outcomes for patients undergoing HD during the ongoing COVID-19 pandemic.

Conflict of interest statement. The authors declare no conflicts of interest.

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The authors' contributions.

N. Stepanova: Conceptualization, data analysis and interpretation, manuscript writing;

V. Driianska: Cytokines measurement;

L. Korol: IS assay;

L. Snisar: Data collection;

S. Savchenko: Clinical laboratory markers measurement.

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