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# Morphofunctional state of the kidneys of laboratory rats during acute respiratory distress syndrome

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**Abstract.** One of the most common complications of acute respiratory distress syndrome is acute kidney injury, the mechanisms of development of which remain not completely clarified. The purpose of this study is to examine morphofunctional changes in the kidneys of rats with induced acute respiratory distress syndrome at various time intervals after modelling the pathology. For the study, 56 healthy sexually mature male rats weighing 200-220 g were used, divided into 7 groups: control, 6, 24 hours, 3, 7, 14, and 28 days of the experiment. Respiratory distress in animals was caused by inhalation of lipopolysaccharide (5 mg/kg body weight). The kidneys of intact rats had a typical histological structure without specific features. Histological changes in the renal parenchyma of rats in the study groups included compaction of Malpighian bodies, damage and desquamation of epithelial cells of the nephron tubules, and the appearance of signs of disseminated intravascular coagulation. A month after the start of the experiment, both pathological changes in the nephrons and restored or preserved structural components of the kidney are observed, which indicates activation of intracellular reparative processes. The expression of TGF-β1 fibrosis marker as well as CD68 panmacrophage marker increased on days 3 and 7 of the experiment. The number of macrophages in the kidney samples remained consistently high until the end of the experiment, while the level of TGF-β1 decreased on day 28, indicating the start of the resolution phase. Biochemical analysis of renal markers showed an undulating course of inflammatory processes in the kidneys of experimental rats. The maximum concentration of creatinine, urea, and uric acid in the blood serum was observed at 24 hours of the experiment, which indicated the onset of acute kidney injury as a complication of respiratory distress. Preclinical examination of morphofunctional changes in the kidneys during acute respiratory distress syndrome will help choose an effective method for treating this pathological condition in humans in the future

**Keywords:** lipopolysaccharide; acute kidney injury; renal tests; histological analysis; immunohistochemistry

## INTRODUCTION

Acute respiratory distress syndrome (ARDS) is a life-threatening condition characterised by insufficient oxygenation and non-compliant or stiff lungs. The disease is associated with damage to the capillary endothelium and diffuse alveolar damage. Studies on the mechanisms and consequences of the development of acute respiratory infections have become particularly relevant during the COVID-19 pandemic. Critically ill patients with an unfavourable prognosis were diagnosed with ARDS, which led to high mortality. However, today there are no effective therapeutic methods to treat this serious condition [1].

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Among the many complications of ARDS, acute kidney injury (AKI) is one of the most common. It is known to cause a rapid deterioration of renal function within a few hours or days [2, 3]. AKI, also known as acute renal failure, is a common clinical disorder that occurs due to certain conditions, such as ischemia/reperfusion injury of the kidney with an acute loss of organ function and a decrease of the renal filtration fraction [4-6].

M. Malek *et al.* [4] note that ARDS provokes an increase in circulating cytokines, chemokines, and activated immunocytes, initiating a pathological cascade that, in turn, leads to the development of AKI. R. Fenoglio *et al.* [5] indicate that AKI is caused by a damage of the renal vascular system and a decrease in glomerular filtration rate due to the release of endothelin factor 1. In more severe cases of nephrotic syndrome, renal ischemia occurs, which reduces the afferent arteriolar blood supply to the glomerulus. Such microvascular lesions, together with inflammatory interstitial fibrosis and epithelial damage, impair the viability of the nephron tubules.

According to the data provided by A. Hosszu *et al.* [6], in humans, the ischemia/reperfusion injury of the kidney leads to the loss of the brush border, the appearance of focal expansion zones in the proximal tubules, and the accumulation of necrotic casts in the distal tubules. Most often, epithelial cells undergo apoptosis or necrosis in very susceptible external medullary areas.

There are various data on the incidence of AKI as a complication of ARDS. According to S.M. Villacrés *et al.* [7], 35% of adult patients with ARDS develop AKI. Therewith, M. Malek *et al* [4] indicated substantially lower rates of AKI in hospitalised patients with ARDS – 7-10%. According to the latest data from the ARDSnet trail meta-analysis, the incidence of AKI as a complication of ARDS was ≈ 24% [8]. Severe renal damage leads to death in almost half of cases, and other devastating long-term consequences of this condition include end-stage renal failure and dialysis dependence [9, 10].

AKI, which complicates ARDS, often indicates a negative prognosis. In the ARDSnet study, the 180-day mortality rate was much higher in those who had AKI compared to those who did not develop this complication (58% and 28%, respectively) [8]. One retrospective study demonstrated an increase in the number of days of artificial lung ventilation (ILV) (10 vs. 7 days) and the duration of switching to independent breathing (41 vs. 21 hours) in patients with ARDS complicated by AKI compared to ARDS alone [11]. A. Panitchote *et al.* [12] concluded that two-thirds of patients with ARDS developed AKI during intensive care, and almost half of these patients progressed to stage III of AKI according to the Kidney Disease: Improving Global Outcomes (KDIGO) classification.

The exact underlying mechanisms of AKI development in patients with ARDS are still unclear and are, therefore, the subject of increased scientific attention. The authors of this study planned a preclinical study of histological changes in the kidneys of rats with ARDS induced in different time periods after modelling the pathology to elucidate the subtle mechanisms of kidney damage and select effective methods for treating AKI during respiratory distress syndrome.

## MATERIALS AND METHODS

56 healthy sexually mature male Wistar rats were used for experiments. At the beginning of the experiment, the average body weight of animals was 200-220 g. Rats were kept under standard vivarium conditions (12-hour day/night cycle; t = 20-25 °C; humidity 40-45%) [13], with free access to water and food. Experiments were conducted in the summer. The work with animals was organised in compliance with the principles of the "European Convention for the protection of vertebrates used for experimental and scientific purposes" [14] and the definition of the first National Congress on Bioethics [15].

ARDS was modelled by intranasal administration of lipopolysaccharide (LPS) (SIGMA-ALDRICH), previously diluted with an isotonic sodium chloride solution (1 mg of LPS in 4 mL of NaCl). Using a nebuliser, animals were given LPS at a dose of 5 mg/kg of body weight for 30 minutes. During inhalation, rats were anaesthetised by a peritoneal injection of ketamine at a dose of 50 mg/kg. The animals were randomly divided into 7 groups (8 animals each). Morphological changes in the kidneys were analysed after 6 hours, 24 hours, 3 days, 7 days, 14 days, and 28 days of the experiment. Animals were removed from the experiment by terminal anaesthesia with sodium thiopental at a dose of 150 mg/kg. ARDS modelling was done in the vivarium of I. Horbachevsky Ternopil National Medical University (TNMU).

For histological analysis, pieces of the left kidney were fixed in 10% neutral buffered formalin. Then the tissues were processed in a LOGOS One histoprocessor (Milestone Medical, USA) and embedded into paraffin blocks. 5 microns thick sections obtained on the AMR400 rotary microtome (Amos scientific, Australia) were stained with hematoxylin and eosin [16].

Immunohistochemical evaluation of the expression of transforming growth factor beta 1 (TGF-β1) and CD 68 panmacrophage marker was performed using recombinant rabbit monoclonal primary antibodies (Cat. No. ab215715; No. ab125212, Abcam, USA, respectively) and Mouse/Rabbit PolyVue™ HRP/DAB detection systems (Diagnostic BioSystems, USA). The tissue sections were deparaffinised and rehydrated. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide. Additionally, the sections were counter-stained with Mayer hematoxylin. Immunohistochemical changes in the kidneys were analysed at 24 hours, 3 days, 7 days, and 28 days of the experiment. These time periods correspond to different phases of ARDS development (acute (24 hours – 3 days), subacute (7 days), and chronic (28 days)) [17]. Histological and immunohistochemical samples were examined under an Eclipse Ci-E light microscope and documented using an M3CMOS 14000 camera (Sigeta, Ukraine) at the laboratory of immunohistochemical and immunocytochemical studies of the experimental sector of the interdepartmental training and research laboratory (ITRL) of TNMU.

Rat blood was taken through a cardiac puncture. The serum was obtained through blood centrifugation and stored at – 80°C until analysis. Serum creatinine, uric acid, and urea levels were determined according to the manufacturer's instructions using Spinreact (Spain) kits. The analyses were conducted on the basis of MNL TNMU.

The study was conducted within the framework of the interdepartmental state-funded comprehensive research project of I. Horbachevsky Ternopil National Medical University "Investigation of the regenerative potential of cellular therapy agents in acute respiratory distress syndrome" (state registration number 0121U100159), 2021-2023.

## RESULTS

Histological examination of the kidneys of intact rats did not reveal any pathological changes. Light microscopy examination of the renal cortical substance in intact rats revealed numerous renal (Malpighian) bodies, in which the urinary lumen of the Bowman capsule was clearly visualised. The proximal convoluted tubules were lined with a single-layer prismatic epithelium with a brush border and basal striation. In the distal convoluted tubules, the epithelium was simple cuboidal without a border with deep basal invaginations of the cell membrane. In the interstitium between the tubules of the nephrons, the lumen of the peritubular capillaries was visible (Fig. 1).

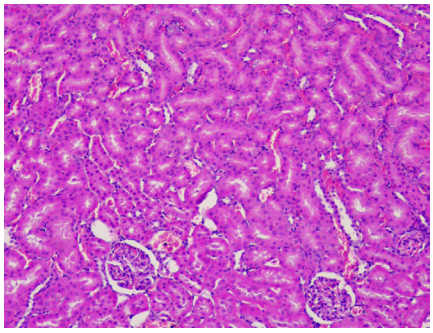

**Figure 1.** Microscopic structure of the cortical substance of the kidney of an intact rat. H&E staining (x200 magnification)
**Source:** photographed by the authors

Moderately blood-filled hemocapillaries of the peritubular network were visible in rats with simulated ARDS after 6 hours of the experiment. The epithelium of the proximal tubules was relatively preserved. There was no oedema. Renal corpuscles were deformed with partially blood-filled capillaries. The lumen of the Bowman capsule was narrowed, compaction of the vascular glomerulus is observed. Damage to the apical surface of distal epithelial cells and an enlarged lumen of the tubules were identified. In the proximal tubules, in some places, desquamation of the epithelium was registered. Dilated and blood-filled venous vessels with clotted lumen, oedematous intima, and spasmodic arteries were observed (Fig. 2).

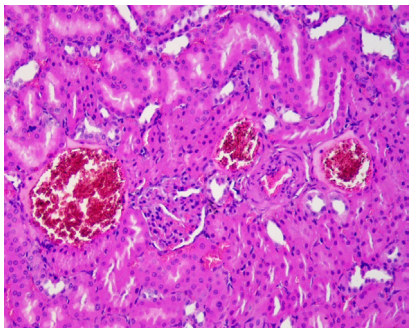

**Figure 2.** Microscopic structure of the rat kidney at 6 hours of the experiment. H&E staining (x200 magnification)
**Source:** photographed by the authors

One day after the start of the experiment, signs of stasis were observed in small calibre vessels. The urinary space of the Bowman capsule was practically not visible, the vascular glomeruli were compacted, the lumen of the nephron tubules was not visible due to the accumulation of cellular debris in the proximal region and oedema of the lining epithelium in the distal region (Fig. 3).

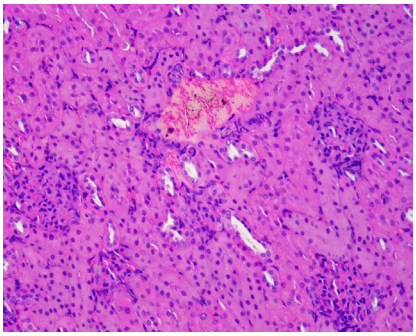

**Figure 3.** Histological structure of the rat kidney on day 1 of the experiment. H&E staining (x200 magnification)
**Source:** photographed by the authors

3 days after LPS administration, dilated blood-filled veins with a damaged wall and signs of disseminated intravascular coagulation (DIC) syndrome were identified. Arteries were also spasmodic. Oedema and homogenisation of epithelial cell cytoplasm were observed in the nephron tubules (Fig. 4). The lumen of the Bowman capsule was not visible, which was obviously an indicator of the impaired functional ability of the glomeruli to filter blood plasma.

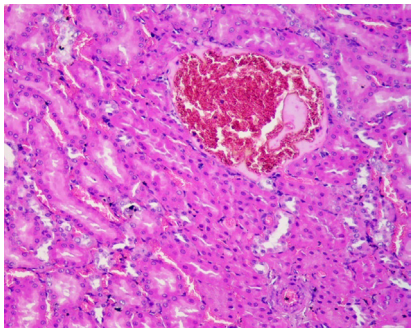

**Figure 4.** Microscopic structure of the rat kidney on the 3rd day of the experiment. H&E staining (x200 magnification)
**Source:** photographed by the authors

On the 7th day of the experiment, blood-filled arterial and venous vessels of the renal vascular system with substantially dilated lumen were observed (Fig. 5A). Renal corpuscles contained compacted vascular glomeruli; desquamated cells were visible in the proximal tubules as a result of a disruption of intercellular contacts in the epithelial lining of the tubule (Fig. 5B). 2 weeks after the start of the experiment, signs of DIC continued to be recorded in the vessels of the cortex and medulla of the organ, and accumulation of degeneratively altered epithelial cells in the lumen of the nephron tubules was detected (Fig. 6).

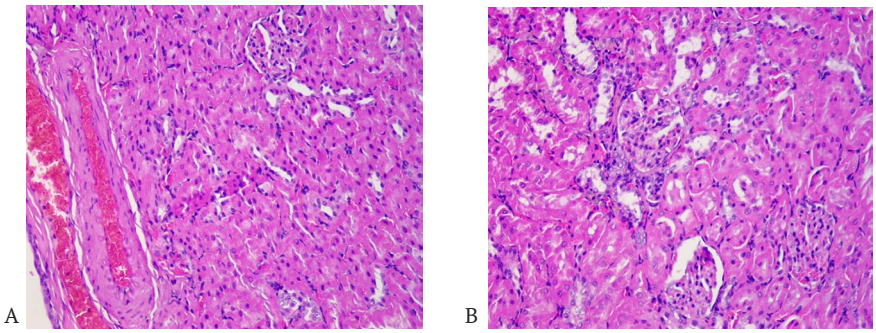

**Figure 5.** Microscopic structure of the rat kidney on the 7th day of the experiment. H&E staining (x200 magnification)
**Notes:** A – signs of blood stasis in the vessels of the renal cortex, B – disruption of contacts between epithelial cells of the proximal tubules
**Source:** photographed by the authors

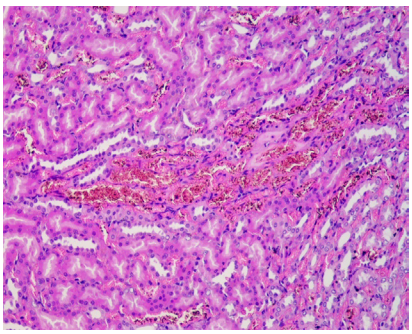

**Figure 6.** Tubulointerstitial lesions of the rat kidney on the 14th day of the experiment. H&E staining (x200 magnification)
**Source:** photographed by the authors

On the 28th day of the experiment, less substantial lesions of the vascular bed were observed, which in some places were manifested by blood filling and stasis. The appearance of oedema of the renal stroma and the dilatation of perivascular spaces were registered. The renal corpuscles were of different sizes. Some of the Malpighian bodies had well-defined vascular glomeruli and the urinary lumen of the Bowman capsule, while irregularly shaped Malpighian bodies with damaged and compacted glomeruli were seen nearby. A number of the proximal tubules of the nephrons contained desquamated epithelium (Fig. 7A, 7B).

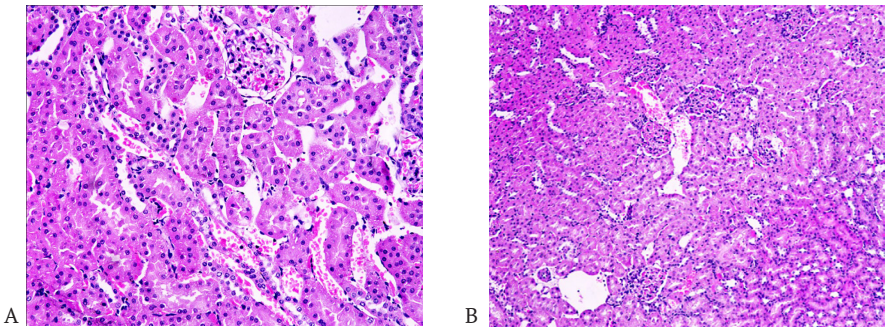

**Figure 7.** Microscopic structure of the rat kidney on the 28th day of the experiment H&E staining
**Notes:** A – x200 magnification; B – x100 magnification
**Source:** photographed by the authors

The development of pathological changes in the renal parenchyma of rats with simulated ARDS occurred gradually. At 6 hours of the experiment, the structure of the nephrons remained relatively unchanged with mild damage to the renal bodies, while at 24 hours, narrowing of the urinary space of the Bowman capsule in the renal bodies appeared, and on the 3rd day and later compaction of the Malpighian bodies indicated impaired plasma filtration. The degree of desquamation of epithelial cells of the nephron tubules also increased with the time of the experiment, and this, in turn, indicated the impairment of the reabsorption of organic components and electrolytes from the primary urine. Therewith, changes in the vascular bed were also documented in the kidneys of

experimental animals. In the initial stages (6-24 hours), small vessels of the kidneys were dilated and filled with blood, while on the 3rd day, signs of DIC appeared in the microcirculatory bed. On day 7 and later, stasis in the renal vessels of various calibres was still observed. One month after the start of the experiment, both degenerated renal corpuscles and desquamated epithelium in the nephron tubules as well as the restored structural components of the kidney were observed, indicating activation of intracellular reparative processes in the resolution phase.

Immunohistochemical detection of TGF-β1 in the kidneys of control rats showed a weak response, mainly in the mesangium of Malpighian bodies (Fig. 8A). After staining the material with CD68 macrophage marker, a similar pattern was observed. In addition, an intense positive staining was identified in the area of large vessels (veins) as well as a slight staining of the interstitial space (Fig. 8B)

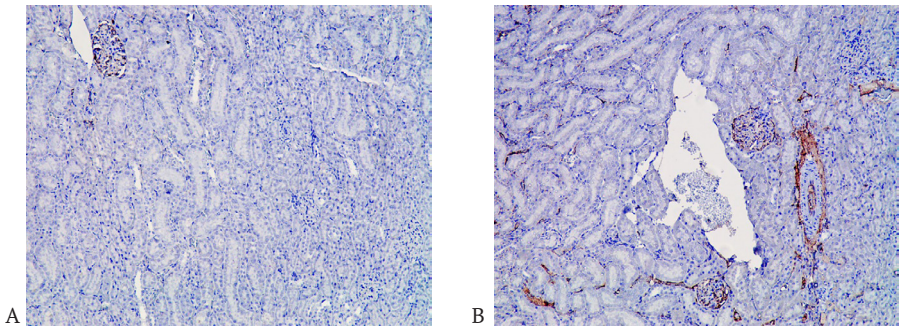


**Figure 8.** Immunohistochemical staining of intact rat kidneys. x100 magnification
**Notes:** A – TGF-β1 staining; B – CD68 staining
**Source:** photographed by the authors

TGF-β1-and CD68-positively stained areas around small calibre vessels are detected in kidney sections of experimental animals 24 hours after ARDS modelling, largely, when stained with TGF-β1. Immunoprecipitation is mostly visible around large vessels (veins). An immunopositive response to TGF-β1 is also observed in the interstitial space (Fig. 9A, 9B).

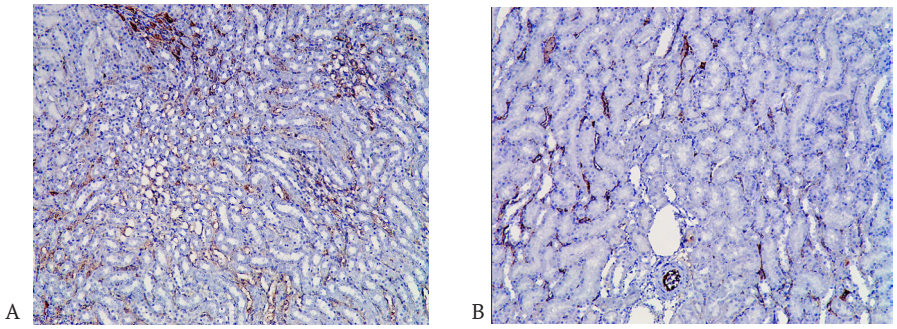


**Figure 9.** Immunohistochemical staining of kidneys on day 1 of the experiment. x100 magnification
**Notes:** A – TGF-β1 staining; B – CD68 staining
**Source:** photographed by the authors

In the kidneys of animals on day 3 of ARDS there was the maximum number of intensely TGF-β and CD68 immunostained perivascular spaces both around the nephron tubules and in Malpighian bodies compared to the control group (Fig. 10A, 10B).

A similar pattern was observed in the kidneys of rats 7 days after the simulated pathology (Fig. 11A). In the case of CD68 staining, a more intense positive staining of the walls of small vessels and intercellular space was also detected at this time point comparing to day 3 of the experiment (Fig. 11B).

On the 28th day of the experiment, mesangium of Malpighian bodies was intensively TGF-β1 immunostained. Immunoprecipitate was also visible although to the lesser extent in the intercellular space of the interstitium (Fig. 12A). CD68 staining revealed a pronounced immunopositive reaction in the vascular areas of the microcirculatory vascular bed and perivascular space (Fig. 12B).

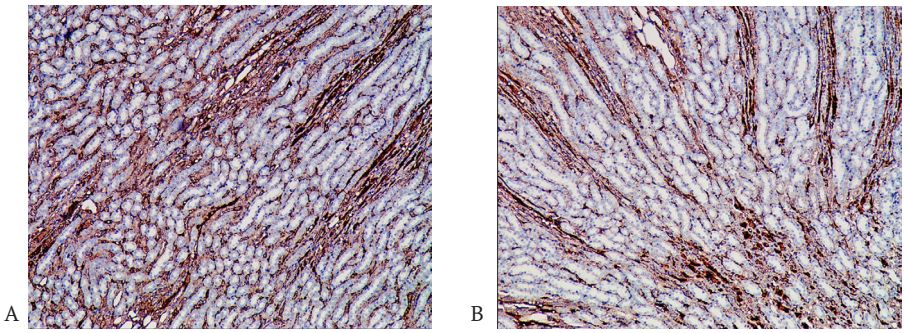

**Figure 10.** Immunohistochemical staining of kidneys on day 3 of pathology. x100 magnification
**Notes:** A – TGF-β1 staining; B – CD68 staining
**Source:** photographed by the authors

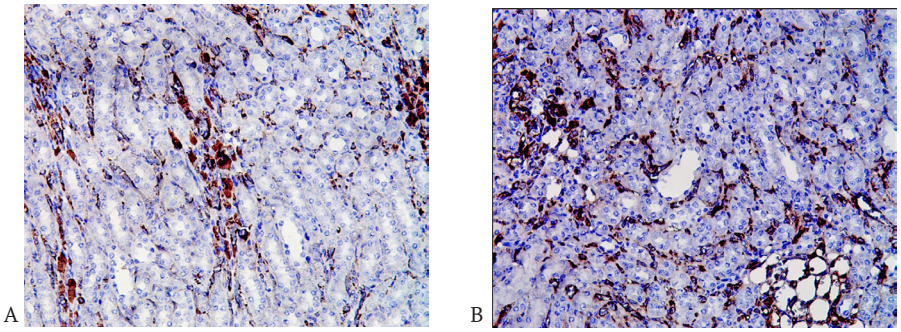

**Figure 11.** Immunohistochemical staining of kidneys on day 7 of ARDS. x200 magnification
**Notes:** A – TGF-β1 staining; B – CD68 staining
**Source:** photographed by the authors

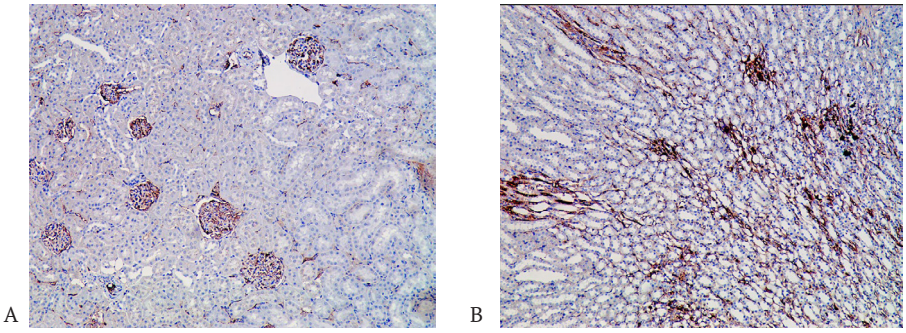

**Figure 12.** Immunohistochemical staining of kidneys on day 28 of ARDS. x100 magnification
**Notes:** A – TGF-β1 staining; B – CD68 staining
**Source:** photographed by the authors

Thus, immunohistochemical analysis of the fibrosis marker TGF-β1 showed an increase in the intensity of immunoprecipitation in kidneys. The most intense expression of TGF-β1 was observed in the subacute stage of ARDS (3-7 days). In the chronic stage (28 days), the intensity of interstitial immunostaining decreased but remained pronounced in the renal corpuscles. This may indicate the development of fibrosis in the mesangia between the capillary loops of the vascular glomerulus. Immunohistochemical detection of the CD68 panmacrophage marker showed an increase in the number of macrophages on day 3 after ARDS modelling, after which the number of these cells remained elevated in the kidney until the end of the experiment.

A biochemical blood test of experimental animals allowed assessing changes in the concentrations of renal markers. The obtained results are shown in the diagrams (Fig. 13A, 13B, 13C).

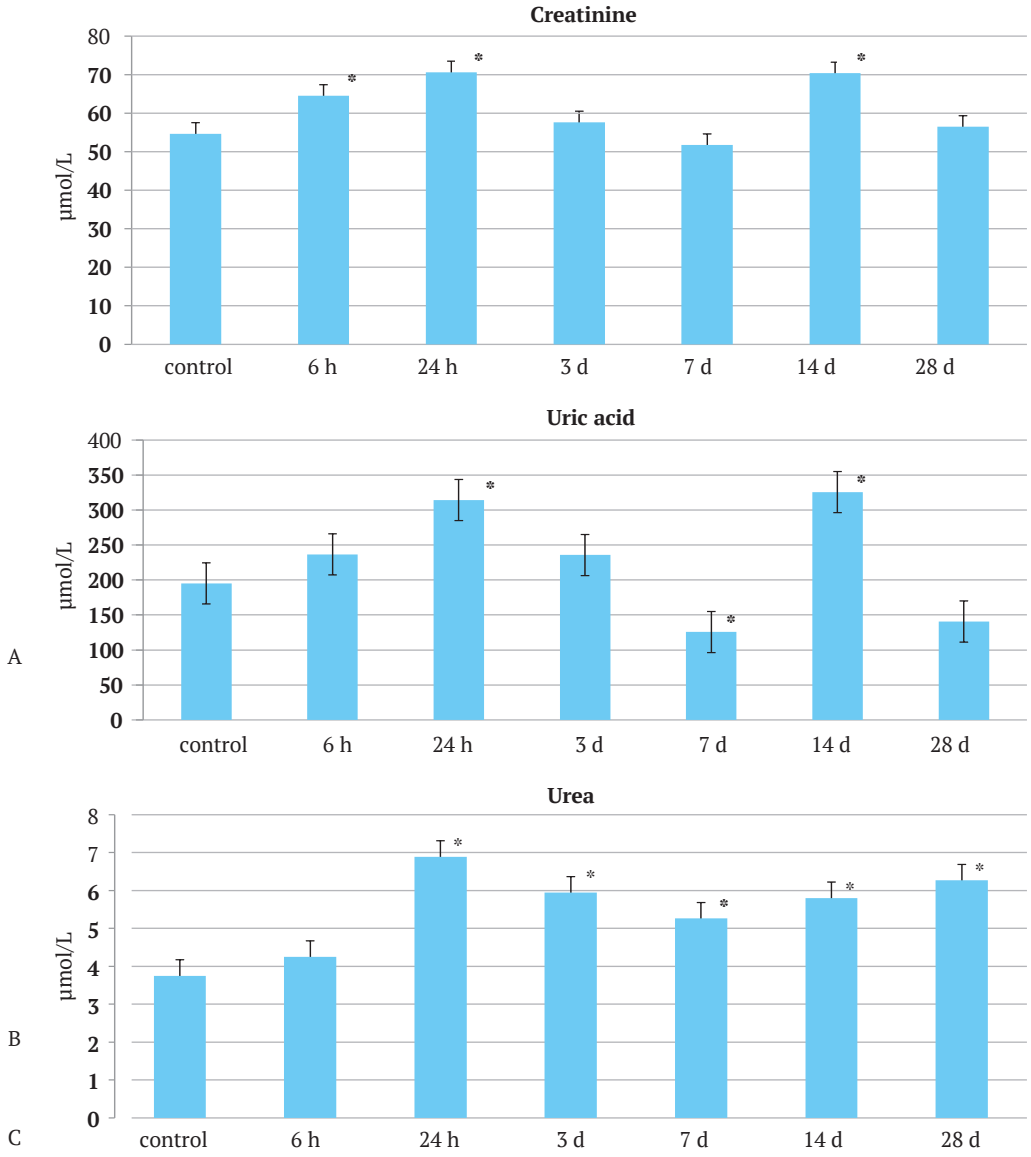


**Figure 13.** Changes in serum renal marker concentrations after ARDS modelling
**Notes:** A – change in creatinine concentration; B – change in uric acid concentration; C – change in urea concentration; * – statistically significant difference compared to the control (P≤0.05)
**Source:** compiled by the authors

Analysis of serum creatinine, uric acid, and urea levels identified their increase in groups of rats with simulated ARDS. In particular, creatinine increased by 1.2 times in the 6th hour compared to the control, reached the first peak at the 24th hour (1.3 times more than the control value), decreased to the control level on the 3rd day, and remained reduced on the 7th day of the experiment. The second peak of creatinine growth was observed on the 14th day (1.29 times higher than the control), and normalisation of the indicator was recorded a month after the onset of pathology development.

An increase in uric acid concentration was observed starting from the 6th hour, with the first peak on the 24th hour (1.6 times higher than the control) and the second peak on the 14th day (1.7 times higher than the control). A statistically significant decrease (1.6 times) in uric acid concentration occurred on the 7th day of the experiment, while on the 28th day of the experiment, the uric acid level was close to the control value. A pathological decrease in uric acid levels 7 days after ARDS modelling may indicate a malfunction of the liver, which leads to its inability to convert amino acids and ammonia into urea.

The level of urea in the blood serum of rats increased slightly at the 6th hour, rapidly peaked at the 24th hour (1.8 times higher than the control value), slightly decreased at the 3rd and 7th days, but remained statistically substantially higher than in intact animals. After 2 weeks and a month from the start of the experiment, the urea concentration remained substantially higher than the baseline level (1.5 and 1.7 times higher, respectively), which indicates the development of chronic kidney damage in laboratory animals.

The results of a biochemical blood test confirm the occurrence of functional disorders of the kidneys caused by the development of simulated ARDS. Undulating changes

in creatinine, urea, and uric acid concentrations indicate that the development of kidney damage was non-linear, with several peaks in the activation of inflammatory processes. The maximum increase in renal marker values in rat blood serum 1 day after ARDS modelling indicates the development of acute kidney injury, which gradually turned into chronic damage.

## DISCUSSION

It is known that ARDS occurs in 3 stages: the first phase is acute, or exudation, the second is subacute or fibroplasia, and the third stage is chronic, fibrosis, or resolution [17, 18]. As shown by the results of histological examination of the kidney and biochemical blood analysis in rats, the acute stage is observed from 6 hours to 3 days of the experiment. The subacute stage corresponds to 7-14 days of experiment, while the third phase occurs a month after the onset of pathology. According to the data obtained, the most pronounced signs of AKI appear on 1-3 days of the experiment.

According to R. Vashisht & A. Duggal [19], AKI is observed in ≈ 45% of patients with ARDS, and on average, its signs start to appear 2 days after the initial diagnosis. ARDS causes impaired gas exchange in the lungs, which leads to the development of hypoxemia, hypercapnia and systemic acidosis, and this, in turn, affects vascular resistance, altering renal perfusion pressure and leading to damage to kidney components [20]. Systemic inflammation, hypoxemia, and ILV are considered to be the three main possible mechanisms of renal damage development in ARDS in humans [18, 21]. High alveolar pressure has been shown to alter the hemodynamics of the heart, leading to hypoperfusion in all organs of the body, especially in the kidneys, leading to a decrease in glomerular filtration rate and the development of AKI [19, 21, 22].

According to the report of the pathomorphological study by M. Shao *et al*. [23], acute renal tubule damage was observed in six cases of patients who died from COVID-19. According to H. Su *et al.* [24], in 26 patients with ARDS developed in severe COVID-19, noticeable acute damage to the proximal tubules of the nephrons was identified on the autopsy, which manifested itself in the form of loss of the brush border, vacuolar degeneration, dilation of the lumen of the tubules with cellular debris, and in some cases outright necrosis and detachment of the epithelium with the exposed basement membrane of the tubules (in 4 patients). Similar morphological signs of kidney parenchyma damage were observed in this study.

In the preclinical study by W. Lv *et al.* [25], AKI was induced by peritoneal administration of LPS. As shown in the histological analysis of changes in the kidneys of rats after modelling the pathology, the structure of the organ in the experimental groups of animals became damaged, oedema of epithelial cells of the renal tubules appeared, as well as the narrowing of the lumen of the tubules, renal tissue was infiltrated by a large number of inflammatory cells in contrast to the kidneys of intact rats. Such pathological changes gradually worsened with the duration of the experiment (8 hours, 12 hours, 24 hours, and 48 hours), and the rate of damage to the renal tubules correspondingly increased. H. Gao *et al.* [26], analysing histological sections of rat kidneys in LPS-induced AKI, identified vacuolar degeneration and damage to the brush border in epithelial cells of the renal tubules. In addition, researchers also observed necrosis of epithelial cells of the nephron tubules.

Examinations of AKI in severe cases of COVID-19 have shown that the main mechanism of this complication is similar to severe sepsis. Acute tubular necrosis in the renal parenchyma was reported in 66% of cases [27]. Cytokine storm caused by ARDS causes hypotension, which leads to decrease of renal perfusion [28]. In addition, focal renal fibrin blood clots were located in histological samples of the kidneys, which appear as a result of systemic coagulopathy. Regardless of the root cause, after the development of acute kidney injury, the intrarenal inflammatory cascade is activated, which, if left unchecked, leads to additional damage and irreversible fibrosis.

According to a number of authors, TGF-β is the main regulator of renal inflammation and fibrosis [29, 30]. TGF-β has a multifunctional effect on cell proliferation, apoptosis, migration, and differentiation. Under the influence of this growth factor, mesenchymal transformation of epithelial cells of the tubules and glomeruli of the kidney is observed, which leads to the development of fibrosis [30]. According to Y. Isaka [31], TGF-β1 is highly expressed in the kidneys during a wide range of fibrosis-related diseases. According to the results of immunohistochemical analysis, TGF-β1 was most strongly detected in the kidneys on the 3rd day of ARDS development. At a later stage of the experiment, a decrease in the expression of this marker indicated the resolution of AKI without the development of pronounced fibrosis. This means that the rats' bodies gradually coped with the inflammatory cascade and managed to start the recovery processes in the damaged kidneys. O. Redko *et al*. [32], in their studies on the effect of ARDS on the morphofunctional state of rat liver, showed that in the early stages of pathology, an increased number of M1 macrophages is observed, which indicates the activation of a pro-inflammatory response in the body, while in the later stages of the disease, M2 macrophages with anti-inflammatory function, and the ability to promote tissue repair begin to elevate. These data are consistent with the results of this study showing that the third stage of development of pathology in the kidneys in ARDS follows the path of resolution, and not fibrosis.

In addition to the fibrosis marker, the expression of CD68 panmacrophage marker, which indicates the total number of macrophages in kidney tissue, was analysed. The obtained data indicate a steady increase in phagocytes in the studied organ, starting from the 3rd day of the experiment. The increased number of macrophages in the late stages of ARDS may be due to the appearance of a large number of anti-inflammatory M2 cells. H. Akdam *et al*. [33] also reported an increase in macrophage count during necrotising glomerulonephritis, which positively correlated with an increase in serum creatinine levels.

Biochemical parameters of creatinine, urea, and uric acid in the blood are important markers of kidney injury [34]. Elevated levels of these renal markers were identified, which increased in two peaks (at 24 hours and on 14 days, respectively). This indicates that LPS successfully induced AKI in rats. Changes in these indicators indicate the development of functional disorders in the kidneys. In particular, elevated serum creatinine levels indicate a disruption of glomerular filtration rate [35-37].

W. Lv *et al*. [25] established a substantial increase in serum creatinine and urea levels 8 hours after toxin injection and a peak value 12 hours later, which was three times higher than the baseline level of the control group. However, levels of these renal markers gradually decreased 24 hours, 48 hours, and 72 hours after LPS administration and returned to baseline levels after 7 days. Interestingly, creatinine increased slightly on day 14 after LPS administration, which corresponds to the stage of transition of AKI to CKD. Such observations are consistent with the results obtained in this study.

F.Z. Khamissi *et al*. [38] exposed mice of the C57BL/6 line to bilateral ischemia/reperfusion kidney injury and analysed signs of AKI. Kidney damage caused a substantial increase in blood urea nitrogen levels on day 1 after the development of AKI, indicating severe renal failure, with a slow improvement of about 50% over 3-5 days. Accordingly, serum creatinine levels were substantially elevated on the first day and remained elevated at a lower level for 3-5 days.

A study by S. Liu *et al*. [39] showed that the development of AKI in sepsis and ARDS is also accompanied by an increase in uric acid levels in the blood of patients. Hyperuricemia promotes damage and apoptosis of vascular endothelial cells by increasing oxidative stress, which increases the formation and elimination of reactive oxygen species, causes endoplasmic reticulum dysfunction, and inhibits the activity of endothelial nitric oxide synthase. In addition, elevated uric acid levels promote the formation of sodium urate crystals in vascular intima, enhance the expression of leukocyte adhesion molecules, and stimulate the production of a large number of associated inflammatory factors, further enhancing endothelial cell dysfunction [39, 40]. Thus, based on the results of histological and histochemical analysis of the kidneys and biochemical blood analysis, it was established that ARDS is accompanied by morphofunctional changes in the kidneys, and this fact is an acute medical problem that needs to be solved by developing an effective treatment method.

## CONCLUSIONS

The simulated ARDS causes substantial changes in rat kidney tissue and its effect manifests itself in different ways, depending on the duration of the experiment. In the initial stages of pathology, damage to the renal bodies with narrowing of the urinary space of the Bowman capsule was noted. Subsequently, signs of stasis developed in the microcirculatory bed and larger vessels, as well as the destruction of epithelial cells of the proximal and distal tubules. At a later stage of the study, both degenerated renal corpuscles and nephron tubules and restored structural components of the kidney were observed.

According to the results of an immunohistochemical examination of TGF-β1 fibrosis marker, it was revealed that the change of immunostaining intensity depended on the duration of the experiment. The most intense expression of TGF-β1 was observed on 3-7 days (subacute phase of ARDS – stage of fibroplasia), afterwards the intensity of immunoprecipitation in the interstitium decreased, which indicated a transition to the resolution stage (28 days). However, fibrotic changes remained pronounced in the renal corpuscles, which indicated the deposition of collagen in the mesangia between the capillaries of the vascular glomerulus. The result of immunohistochemical detection of CD68 total macrophage marker showed an increase in the number of macrophages on the day 3 of the experiment, after which their level remained elevated in the kidney until the end of the experiment.

Biochemical analysis of renal markers in rat blood serum confirmed the presence of functional renal disorders caused by the development of simulated ARDS. The recorded maximum increase in creatinine, urea, and uric acid values at 24 hours from the start of the experiment indicates the development of an acute phase of kidney damage, which gradually turned into chronic damage. Undulating changes in the levels of renal markers indicate a nonlinear course of inflammatory processes in the parenchyma and stroma of the kidneys.

Histological, immunohistochemical, and biochemical analyses of rat kidney damage form an overall picture of morphofunctional changes in this organ during simulated ARDS. The obtained data will contribute to assessing the nephroprotective effectiveness of the latest treatment agent, the therapeutic potential of which is planned to be studied at the next research stage.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## Морфофункціональний стан нирок лабораторних щурів за умов гострого респіраторного дистрес-синдрому

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**Анотація.** Одним з найчастіших ускладнень гострого респіраторного дистрес-синдрому є гостре ураження нирок, механізми розвитку якого залишаються до кінця нез'ясованими. Метою даної роботи було вивчення морфофункціональних змін у нирках щурів з індукованим гострим респіраторним дистрес-синдромом у різні відтинки часу після моделювання патології. Для дослідження використано 56 здорових статевозрілих щурів-самців, масою 200-220 г, яких розподілили на 7 груп: контрольна, 6 та 24 год, 3, 7, 14 та 28 діб експерименту. Респіраторний дистрес у тварин викликали за допомогою інгаляторного введення ліпополісахариду (5 мг/кг маси тіла). Нирки інтактних щурів мали типову гістологічну будову без видових особливостей. Гістологічні зміни паренхіми нирок щурів дослідних груп включали ущільнення мальпігієвих тілець, пошкодження та десквамацію епітеліоцитів канальців нефронів та появу ознак дисемінованого внутрішньосудинного згортання крові. Через місяць від початку експерименту спостерігалися як патологічні зміни в нефронах, так і відновлені чи збережені структурні компоненти нирки, що вказувало на активацію внутрішньоклітинних репаративних процесів. Імуногістохімічно встановлено зростання експресії маркера фіброзу TGF-β1 та підвищення інтенсивності імунозабарвлення препаратів за панмакрофагічним маркером CD68 на 3 і 7 доби досліду. Кількість макрофагів у зразках нирки залишалась стабільно високою до кінця експерименту, в той час як рівень TGF-β1 знижувався на 28 добу, вказуючи на розвиток фази розрішення патології. Біохімічний аналіз ниркових маркерів показав хвилеподібний перебіг запальних процесів у нирках дослідних щурів. Максимальна концентрація креатиніну, сечовини і сечової кислоти в сироватці крові спостерігалась на 24 год експерименту, що свідчило про настання гострого ураження нирки як ускладнення респіраторного дистресу. Доклінічне вивчення морфофункціональних змін нирок за умов гострого респіраторного дистрес-синдрому допоможе в майбутньому підібрати ефективний метод корекції даного патологічного стану у людей

**Ключові слова:** ліпополісахарид; гостре ураження нирок; ниркові проби; гістологічний аналіз; імуногістохімія