

# The effects of prolonged CO<sub>2</sub> insufflation on kidney function in a rat pneumoperitoneum model

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## Abstract

**Introduction:** Pneumoperitoneum (PP) is known to cause ischemia in kidneys and other intra-abdominal organs because of decreased splanchnic blood flow.

**Aim:** We aimed to determine the degree of renal injury that occurs due to a PP and prolonged PP. We measured renal injury biomarkers and made a histopathological evaluation to estimate the degree of injury and assessed the correlation of biomarkers with histopathological findings.

**Material and methods:** Twenty-one female Sprague Dawley rats were separated randomly into three groups. Group 1 was the control group and was given anesthesia for 3 h. In group 2, a PP was administered under anesthesia for 1 h. A pneumoperitoneum was administered under anesthesia to animals in group 3 for 3 h.

**Results:** Pathological analysis showed a significant statistical difference between the 3 groups. In particular, neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C (Cys C) levels at the 24<sup>th</sup> h and preoperative mean urea levels showed a significant difference between the groups. The 24<sup>th</sup>-hour NGAL level in group 3 was significantly higher than that of group 1. The preoperative Cys C level was higher in group 1 than in either group 2 or 3. Cys C was decreased significantly in group 1 and increased significantly in both groups 2 and 3.

**Conclusions:** The increase in NGAL and Cys C levels directly correlated with the duration of PP and intra-abdominal pressure, and they are therefore good biomarkers in diagnosing acute renal injury in the early phase. Serum creatinine level is not a good biomarker in the early phase of renal injury.

**Key words:** pneumoperitoneum, neutrophil gelatinase-associated lipocalin, cystatin C, acute kidney injury, rats.

## Introduction

Laparoscopic surgery with a CO<sub>2</sub> pneumoperitoneum (PP) has become a widely used technique [1]. It is currently an accepted approach that is beneficial for patients, dramatically shortens operative

time and morbidity, and has a better postoperative recovery period. But, as would be expected, it has some disadvantages: decreased venous return, subcutaneous emphysema, impaired ventilation, a risk of gas emboli and, especially in small mammals, abdominal compartment syndrome due to increased

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intra-abdominal pressure (IAP) [2–4]. The PP has been known to cause ischemia in kidneys and other intra-abdominal organs because of decreased splanchnic blood flow [5, 6]. Experimental studies have shown that oliguria occurs due to an IAP-related decrease in renal blood flow, urinary output, and glomerular filtration rate (GFR). Vascular compression, ureteral obstruction, systemic hormonal effects, and direct renal compression-related decrease in renal blood flow have been proposed as possible causes of oliguria [2–4, 7, 8].

Acute kidney injury (AKI) is diagnosed based on elevated levels of serum creatinine (SCr) or the duration and severity of oliguria [9]. However, the level of serum creatinine is not a proper biomarker of renal tubular injury, but rather an indicator of the balance between creatinine production and excretion. Nonetheless, there is no difference between the terms the Acute Kidney Injury Network (AKIN) and Kidney Disease Improving Global Outcomes (KDIGO) [9]. The AKI is described as a reversible decrease in GFR due to intrarenal events such as prolonged ischemia and sepsis causing tubular or glomerular cell necrosis, resulting in increased levels of creatinine in the plasma and a temporary decrease in renal blood flow. An increase in SCr might not even be seen in patients with decreased muscle mass who suffer renal injury. Serum creatinine is not a sufficiently specific or sensitive biomarker to indicate nephron injury [10]. Recent studies have shown that a period of increase in SCr levels is insufficient to predict mortality and morbidity [11]. Neutrophil gelatinase-associated lipocalin (NGAL), a 23 kDa protein, is an early biomarker of AKI [12]. In normal circumstances, it is produced in renal cells in small amounts, but its production is regulated in the thick ascending limb (TAL) of the loop of Henle and the collecting ducts, and its level increases in urine and plasma depending on the duration and severity of the injury [13, 14].

The NGAL is a very useful biomarker for early diagnosis of AKI in children with renal implants and in adults undergoing cardiac surgery. Additionally, NGAL can be used as an effective predictor and biomarker for AKI [15, 16]. Changes in levels of NGAL were found to be more effective than those of SCr in predicting the degree of early or subclinical renal injury, the need for dialysis, and mortality [10].

Recently, cystatin C (Cys C), which is superior to SCr in detecting renal injury, was discovered [17, 18]. Cys C is a 13 kDa protein produced by all nucleat-

ed cells and filtered by the glomerular membrane without free secretion. Its plasma concentration is found by using GFR. Cys C is less dependent on any factors (especially gender, age, muscle mass, or age) other than renal function. In intensive care unit (ICU) patients, the reliability of Cys C was tested in GFR assessment. However, cystatin C had limited value in trauma patients [19, 20].

## Aim

We aimed to detect the degree of renal injury caused by a PP and prolonged PP. We measured renal injury biomarkers and made a histopathological evaluation to determine the degree of injury and compared the correlation of the biomarkers with the histopathological findings.

## Material and methods

### Study design

The study was conducted on 21 female Sprague Dawley rats, which were 5 to 6 months old and weighed 300 to 350 g. They were randomly separated into three groups. Group 1 was the control group and was given anesthesia for 3 h. Group 2 was administered a pneumoperitoneum (PP) under anesthesia for 1 h, and group 3 was administered a pneumoperitoneum under anesthesia for 3 h. The study was approved by the Turkish Medicines and Medical Devices Agency (TMMDA) and the Local Ethical Committee on Animal Experiments.

The subjects were kept in standardized laboratory conditions of 20–24°C, 50–60% relative humidity, controlled light (day–night cycle of 12 h : 8/20 h), fed on standardized rodent food, and given filtered and chlorinated water.

The animals were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg).

A 10 mm Hg CO<sub>2</sub> PP was created through a catheter inserted in the left lower abdomen using a Sopro 640 pneumatic 30 L insufflator (Sopro comeq S640-3005, Germany). The PP pressure was set using an intraabdominal catheter with a valve system in groups 2 and 3. This system was composed of a chest drainage bottle, filled with water to form a water column length up to the desired PP pressure level, connected to the insufflator and the rat with a three-way stopcock which allows gas to escape if

this pressure is exceeded. The same pressure was used in all groups.

The NGAL and Cys C measurements were made in blood samples taken preoperatively as well as at the 1<sup>st</sup> and 24<sup>th</sup> postoperative hours; creatinine levels in serum and in urea were measured preoperatively and at the 24<sup>th</sup> postoperative hour. The rats were sacrificed at the 24<sup>th</sup> h and a histopathological analysis of the kidneys was performed.

### Tissue sampling and histopathological examination

The kidneys were fixed in a 10% formaldehyde solution. The cut sections were examined for completeness and one representative section of each kidney was selected for tissue processing. The samples were cut into slices, stained with hematoxylin-eosin dye and examined under a light microscope by a pathologist who was blind to the study design.

All of the sections were examined for evidence of tissue injury. The results were evaluated using the EGTI scoring (\*) system [21]. Tubules, glomeruli, tubulointerstitial area, and endothelial cells were evaluated, and the degree of injury was scored from 0 to 4.

### Biochemical analysis

The creatinine concentration in the serum (SCr) was determined by an enzymatic assay (Creatinine Plus, Roche Diagnostics GmbH, Mannheim, Germany). Plasma NGAL was determined by ELISA (Elisa Kit 036, BioPorto Diagnostics A/S, Gentofte, Denmark). The Cys C concentration was assessed using rat Cys C ELISA (Elisa kit, BioVendor, England). Serum samples for the Cys C measurement were collected and frozen at -80°C until the analysis was carried out. All laboratory investigators were blind to each rat's clinical information, and all measurements were repeated twice to eliminate false results.

### Statistical analysis

SPSS 15.0 for Windows was used for statistical analysis. The descriptive statistics for categorical variables were given as the number, percentage, mean and standard deviation, and for numerical variables as the median. The categorical variables for three groups were compared using the  $\chi^2$  test. When the conditions were not satisfied, Monte Carlo simulation was used. As the parametric test requirement was not present, the comparison of the numerical

variables between independent groups was made using the Kruskal-Wallis test. Subgroup comparison was performed with the Mann-Whitney *U* test and interpreted using Bonferroni correction. Numerical variables were compared with the Wilcoxon test between two groups and with Friedman analysis in comparing more than two independent groups. Subgroup analysis was performed with the Wilcoxon test and interpreted using Bonferroni correction. Statistical significance was assumed for  $p < 0.05$ .

### Results

Results of pathological analysis showed statistically significant differences between the groups. Total scores were significantly different between all the groups. The total mean score of group 1 was the lowest and that of group 3 the highest (Table I).

The NGAL and Cys C levels at the 24<sup>th</sup> h and preoperative mean urea levels in serum showed significant differences between the groups ( $p = 0.019$ ,  $p = 0.002$ ,  $p = 0.003$ ,  $p = 0.032$ , respectively). The NGAL at the 24<sup>th</sup> h in group 3 was significantly higher than that of group 1 ( $p = 0.018$ ). The increase in NGAL was significant in group 3. The differences across all parameters were significant between all the groups ( $p = 0.018$ ). Preoperative Cys C level was higher in group 1 than in groups 2 and 3 ( $p = 0.002$ ,  $p = 0.009$ ). The change of Cys C was downward in group 1 and upward in groups 2 and 3 and statistically significant ( $p = 0.001$ ,  $p = 0.012$ ,  $p = 0.001$ , respectively). All parameters analyzed were significantly different between groups 1 and 3 ( $p = 0.018$  for all). The increase in all parameters for group 2 at the 1<sup>st</sup> h compared to preoperative values was statistically significant ( $p = 0.018$ ). The increase in postoperative urea levels in group 3 and the decrease in creatinine in group 2 were statistically significant ( $p = 0.028$  for both) (Table II).

### Discussion

Laparoscopy is a minimally invasive surgical technique, advantageous compared to open techniques. But it might result in some IAP-dependent side effects, along with its surgical complications. Cases of severe oliguria following laparoscopic surgery were reported and are thought to be due to increased intra-abdominal pressure which results in increased pressure on the renal parenchyma, renal artery, and ureters, leading to decreased cardiac

**Table I.** Histopathologic analysis scores in the three groups

Parameter	Group 2		Group 1		Group 3		P-value
	Mean ± SD	Med.	Mean ± SD	Med.	Mean ± SD	Med.	
Total score	2.50 ± 0.84	2	0.86 ± 0.69	1	5 ± 0.82	5	<b>&lt; 0.001</b>
	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	
Tubular:							
0	0	0.0	1	14.3	0	0.0	<b>0.006</b>
1	6	100	6	85.7	2	28.6	
2	0	0.0	0	0.0	3	42.9	
3	0	0.0	0	0.0	2	28.6	
Endothelial:							
0	0	0.0	6	85.7	0	0.0	<b>0.001</b>
1	6	100	1	14.3	7	100	
Glomerular:							
0	4	66.7	7	100	0	0.0	<b>&lt; 0.001</b>
1	1	16.7	0	0.0	0	0.0	
2	1	16.7	0	0.0	7	100	
Tubulointerstitial:							
0	6	100	7	100	7	100	–
		Group 1 vs. 2		Group 2 vs. 3		Group 1 vs. 3	
		<i>P</i> -value		<i>P</i> -value		<i>P</i> -value	
Total score	<b>0.005</b>		<b>0.003</b>		<b>0.001</b>		

*P*-values in bold are significant ( $p < 0.05$ ).

**Table II.** Change of NGAL, Cys C, serum urea and serum creatinine (SCr) in the three groups

Parameter		Group 1		Group 2		Group 3		<i>P</i> -value
		<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	
		Mean ± SD	Med.	Mean ± SD	Med.	Mean ± SD	Med.	
NGAL	Preop.	7.72 ± 0.80	7.94	6.89 ± 1.03	7.16	6.50 ± 1.29	6.65	0.145
	1 h	7.57 ± 1.49	8.3	7.15 ± 0.66	7.18	7.65 ± 1.39	8.25	0.358
	24 h	7.53 ± 1.04	8.07	7.22 ± 0.18	7.34	9.10 ± 1.20	9.68	<b>0.019</b>
	<i>P</i> -value	0.651		0.867		<b>0.001</b>		
Cys C	Preop.	14.13 ± 1.84	13.51	9.52 ± 1.32	9.5	10.79 ± 1.60	10.98	<b>0.002</b>
	1 h	11.54 ± 1.93	11.6	12.26 ± 0.82	12.47	12.93 ± 1.91	12.27	0.496
	24 h	10.35 ± 1.54	10.23	13.17 ± 2.05	13.46	14.92 ± 1.55	14.24	<b>0.003</b>
	<i>P</i> -value	<b>0.001</b>		<b>0.012</b>		<b>0.001</b>		
Serum urea	Preop.	72.46 ± 21.89	72.3	47.86 ± 9.80	46.2	46.07 ± 2.85	46.9	<b>0.032</b>
	Postop.	55.01 ± 7.12	54.5	52.27 ± 6.81	54.7	61.51 ± 10.84	60.7	0.262
	<i>P</i> -value	0.091		0.176		<b>0.028</b>		
SCr	Preop.	0.56 ± 0.22	0.49	0.52 ± 0.14	0.48	0.47 ± 0.19	0.42	0.464
	Postop.	0.40 ± 0.10	0.38	0.43 ± 0.09	0.42	0.40 ± 0.07	0.39	0.469
	<i>P</i> -value	0.176		<b>0.028</b>		0.612		

*P*-values in bold are significant ( $p < 0.05$ ).

output and increased renin-angiotensin-aldosterone system hormonal activity, though no adverse effect of PP on gastrointestinal system function was found in a study conducted by Cai *et al.* [1, 8, 22–24].

The AKI was reported following laparoscopic surgery in patients with chronic renal failure and in patients requiring renal replacement therapy following operations. Renal failure was considered to be a secondary effect of PP, the main effects of which are on cardiovascular, respiratory, neuro-endocrine, and metabolic systems [25]. The compressive effect of IAP on central veins results in decreased renal perfusion and, therefore, decreased urinary output perioperatively [26]. The IAP-dependent renal vasoconstriction results in increased sodium excretion in the urine and renal tubular dysfunction. Prolonged IAP and operation time increase the risk of renal tissue injury and the development of postoperative cardiovascular complications [1, 27]. Two studies reported such AKI in 2 patients: one 69-year-old man and one 19-year-old woman without any comorbid disease following a laparoscopic cholecystectomy [26, 28].

Kimberley *et al.* carried out a meta-analysis of PP studies in which they analyzed kidney functions in large numbers of animal series in 2016. They reported that PP-induced increases in the SCr level occurred within the first 24 h. The SCr level was found to be normalized within days and weeks. However, this finding was not valid for studies using high pressures and for those concerned with other parameters of renal function. This meta-analysis did not reveal any significant difference in the SCr level according to the gases used for the PP. However, SCr levels were found to be higher in studies that used higher pressures and in studies with prolonged PP duration (> 240 min) [29]. Another similar controlled randomized study reported that SCr showed an increase at the end of the operation compared to its preoperative level, but it started to decrease immediately, as could be seen at the 2<sup>nd</sup> and 24<sup>th</sup> h [30]. Nguyen *et al.* compared perioperative renal function and urinary output between open and laparoscopic surgery in gastric bypass patients and reported that although urinary output decreased significantly in the laparoscopic group, blood urea nitrogen and creatinine levels, which also indicate renal function, did not show any significant change in both groups [31].

McDougall *et al.* reported a decrease in urinary output at PP pressures of 15 mm Hg or more and in

prolonged PP due to decreased renal perfusion, but no renal injury or histopathological change in animals. They also found a significant decrease in SCr [8].

We found a significant decrease in SCr, too, in agreement with other studies. While this decrease was greater in the control group, it was the lowest in the 3-hour PP group. As seen in other studies, SCr level is not a good parameter for indicating the early phase of acute renal injury (AKI).

The NGAL is a biomarker which indicates AKI in its early phase. It is an important protein biomarker that increases within 2 h following ischemic renal injury; it is produced by neutrophils and other epithelial cells (including renal proximal tubular cells). The AKI is a frequent and serious situation observed following cardiopulmonary bypass and cardiac operations. This study analyzed the value of newly introduced biomarkers NGAL and Cys C in prediction of early AKI following cardiac operations. It was found that NGAL level started to increase at the 6<sup>th</sup> postoperative hour and it was higher at the 24<sup>th</sup> h [32]. They analyzed 24 studies in this meta-analysis and decided that serum and urinary levels of NGAL were good biomarkers in diagnosis of AKI which developed following cardiac surgery [33]. We aimed to find the role of plasma NGAL in the assessment of AKI risk within the first postoperative week and found that following implantation, mean NGAL levels were significantly higher in AKI-developed patients.

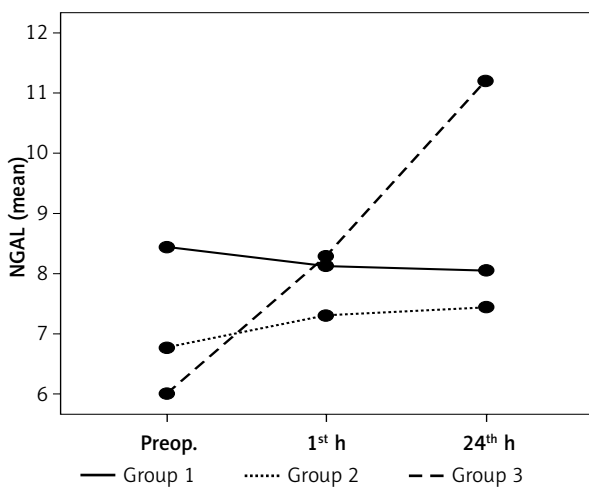
Nickolas *et al.* analyzed patients with AKI, prerenal azotemia, chronic renal disease and normal kidney function. They found that NGAL levels were only high in the AKI group and the SCr level was high only in AKI, prerenal azotemia and, to a lesser extent, in patients with chronic renal failure [10].

De Barros *et al.* found that PP did not change NGAL levels or urinary output in 1 or 2 h PP animals compared to a control group, but urinary output was reduced and NGAL levels were increased in those given cisplatin. Pathological changes, ranging from focal lesions to injuries in a diffuse pattern, were not found to be different from those of the control group either and were instead related to the operation or the method of kidney preservation [34].

Silberstein *et al.* laparoscopically induced unilateral acute renal ischemia in pigs by hilar clamping at certain intervals and only performed a laparoscopy in the control group. They found that NGAL levels in the urine increased in the renal ischemia group at the 24<sup>th</sup> and 48<sup>th</sup> postoperative hours, but the increase

in plasmatic NGAL at the 48<sup>th</sup> h was not significantly different from that of the control group [35].

There are many studies on NGAL in AKI, but few have analyzed IAP due to PP-related AKI. We measured NGAL levels to evaluate IAP-dependent AKI preoperatively, as well as at the 1<sup>st</sup> and 24<sup>th</sup> postoperative hours. There was no difference between NGAL levels in the control group. There was an increase in NGAL level in group 2, although it was not statistically significant ( $p = 0.867$ ). The increase in NGAL level in group 3 was much higher than in the control group at the 24<sup>th</sup> h and significant ( $p < 0.001$ ). When subgroup analysis was performed, there was no significant difference between NGAL levels in the three groups at the 1<sup>st</sup> postoperative hour, but there was at the 24<sup>th</sup> h. We surmised that the longer the PP period was, the greater was the increase in the levels of NGAL, thus indicating greater renal injury. Plasma NGAL was decreased in the control group but increased in the 1 h and 3 h PP groups. This increase was larger in the 3-hour PP group, and there was a significant difference between preoperative and postoperative 1<sup>st</sup> and 24<sup>th</sup> h ( $p < 0.001$  and  $p < 0.001$  respectively). Although there was an increase in NGAL levels in the 1 h PP group, the change between the preoperative and the 1<sup>st</sup> and 24<sup>th</sup> postoperative hours was not significant ( $p = 0.867$ ). There was a significant difference between the values at the 24<sup>th</sup> hour, comparing the three groups ( $p = 0.019$ ). However, there was no significant difference between the preoperative and postoperative 1<sup>st</sup> h values (Figure 1). We related this to the fact that



**Figure 1.** The relationship between NGAL level and PP duration

NGAL levels start to increase 2 h following renal injury and show an increase in correlation with degree of injury at the 24<sup>th</sup> h. Also we considered that since prolonged IAP in the 3 h PP group increased the degree of renal injury, the greatest increase in NGAL level was in this group.

The use of the biomarker Cys C – which is not affected by gender, age or muscle mass – for the evaluation of acute kidney injury has been increasing [17, 18]. Several biomarkers and laboratory tests can be used to assess GFR and tubular function [36, 37]. Changes in SCr are not specific to injury type and not especially sensitive to mild changes in GFR [38, 39]. The level of serum Cys C changes only in AKI. Neither the etiology of AKI nor urine volume has any effect on the predictive value of serum Cys C in AKI. Herget-Rosenthal *et al.* reported that Cys C was superior to creatinine in detecting AKI in their clinical study. They proposed that Cys C could show AKI 1 to 2 days earlier than serum creatinine, which could be useful in preventing the progression of AKI and its harmful effects [40].

Although Cys C has been put forward as an alternative to SCr for estimating GFR, its clinical use has not been growing because of the small number of studies. Lagos-Arevalo *et al.* also showed that Cys C is an important biomarker for diagnosing AKI earlier in non-cardiac pediatric patients admitted to the PICU (where AKI is most common in children’s hospitals) [41].

There are also few studies on Cys C in renal injury following laparoscopic surgery. Lima *et al.* showed that Cys C is a reliable biomarker when assessing changes in GFR and diagnosing renal injury earlier in patients who underwent laparoscopic surgery. Despite not finding any change in SCr level, they found an increase of 4% to 9% in Cys C levels in detecting PP-dependent renal injury [42].

Ozturk *et al.* measured Cys C, urea, SCr and made a histopathological evaluation in order to investigate the useful effect of theophylline in AKI in rats and found that Cys C was lower in the theophylline group, which correlated with the histopathologically determined degree of injury [43].

Mysliwiec *et al.* used different formulas to calculate eGFR in morbidly obese patients and found that formulas based on creatinine were not reliable to estimate GFR but formulas based on Cys C could indicate 2<sup>nd</sup> and 3<sup>rd</sup> stages of chronic renal disease 5 times more reliably, and they suggested using Cys C in renal function evaluation in morbidly obese patients [44].

Gómez Dammeier *et al.* found that urinary output decreased 45 min following PP in infants and children. Out of 8 children younger than 1 year, 7 (88%) developed anuria vs. 3 out of 22 (14%) children aged 1 to 15 years ( $p < 0.001$ ). Nine children of 1 year and older (32%) developed oliguria. There was a significant recovery in the mean urine output by 5 to 6 h after pneumoperitoneum in both age groups. Changes in Cys C, urea, SCr and urine output became more evident at the 24<sup>th</sup> postoperative hour [45].

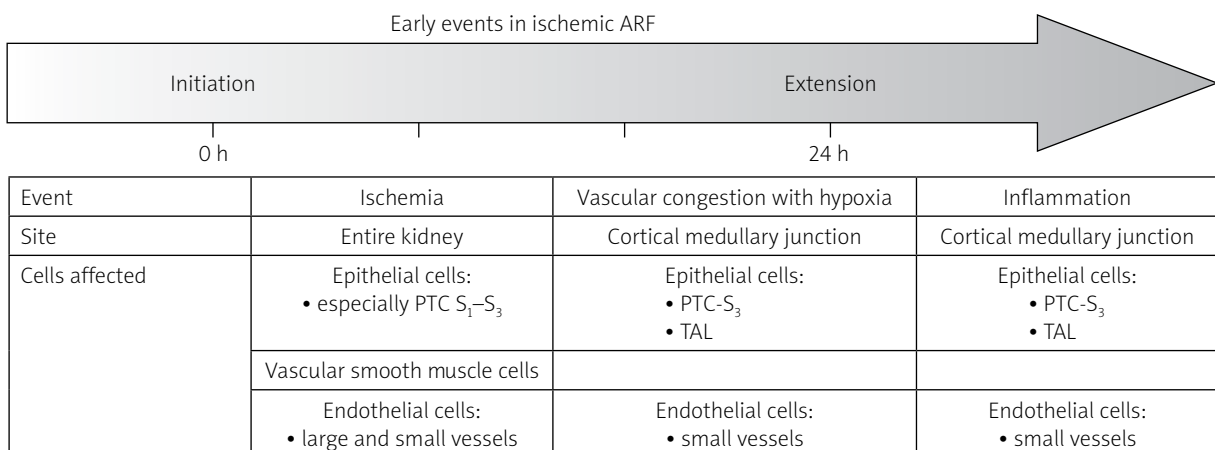
We obtained similar results to other studies. While Cys C level decreased in the control group, the 1 h and 3 h PP groups had elevated Cys C levels. The changes in Cys C levels in all three groups were statistically significant ( $p < 0.001$ ,  $p < 0.012$ ,  $p < 0.001$ , respectively). The increase of Cys C in the 3 h PP group was found to be the highest when comparing the groups in terms of Cys C levels at the 24<sup>th</sup> hour. This result led us to believe that Cys C might be a reliable biomarker in the detection of AKI during the early period.

A decrease in renal blood flow is essential in the pathophysiology of ischemic AKI. Under physiological conditions, the oxygen tension in the kidney decreases from the cortex to the medulla [28]. Interestingly, studies have shown that regional alterations in renal blood flow persist after the initial ischemic event, which may play an important role in the extension phase of renal ischemic injury. A decrease in renal perfusion of between 40% and 50% of normal levels has been reported in both animal and human

models of ischemic AKI during reperfusion following ischemia [46]. Studies have shown that a persistent reduction in renal blood flow contributes significantly to the decreased GFR in human renal allografts following ischemic AKI [47]. These persistent perfusion deficits occur more frequently in the outer medulla than in the outer cortex or inner medulla in animal models of ischemic AKI [48, 49].

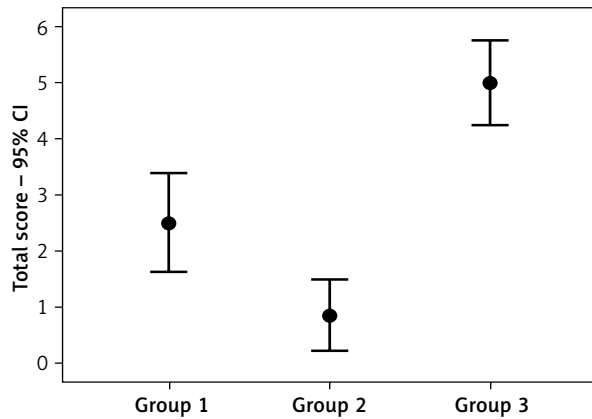
Congestion of renal microcirculation, especially in the peritubular capillaries of the outer medullary region (vasa recta), results in a decrease in renal perfusion. Red blood cells and leukocytes in the outer medulla accumulate, which was shown in both animal models and human ischemic AKI [48, 50, 51]. This medullary congestion has been proposed to shunt blood flow away from the outer medulla, resulting in continued hypoxia and cellular injury in this area. Early findings of ischemic AKI are shown in Figure 2 [52]. Silberstein *et al.* induced unilateral ischemia under PP in pigs and observed mild tubular necrosis and tubular regeneration as a result of ischemic injury; they did not observe any such damage in the control group without ischemia [35].

Khoury *et al.* studied the degree of renal injury for different pressures of PP in mice. While finding nothing at 3 mm Hg of pressure, they found glomerular lobulation and microcalcification at 15 mm Hg and moderate acute tubular necrosis at 18 mm Hg [53]. Khoury *et al.* also reported that oxidizing agents, which are formed due to PP-dependent renal ischemia, cause apoptosis and thus renal injury in mice [54].



**Figure 2.** Early events in ischemic acute renal failure. The initial ischemic insult results in morphological and functional alterations in the renal tubules and the renal vasculature. Following the initial ischemic insult, further alterations in the renal vascular endothelium of the cortico-medullary junction contribute to inflammation and vascular congestion. These processes are proposed to extend the initial injury to the renal tubules [52]

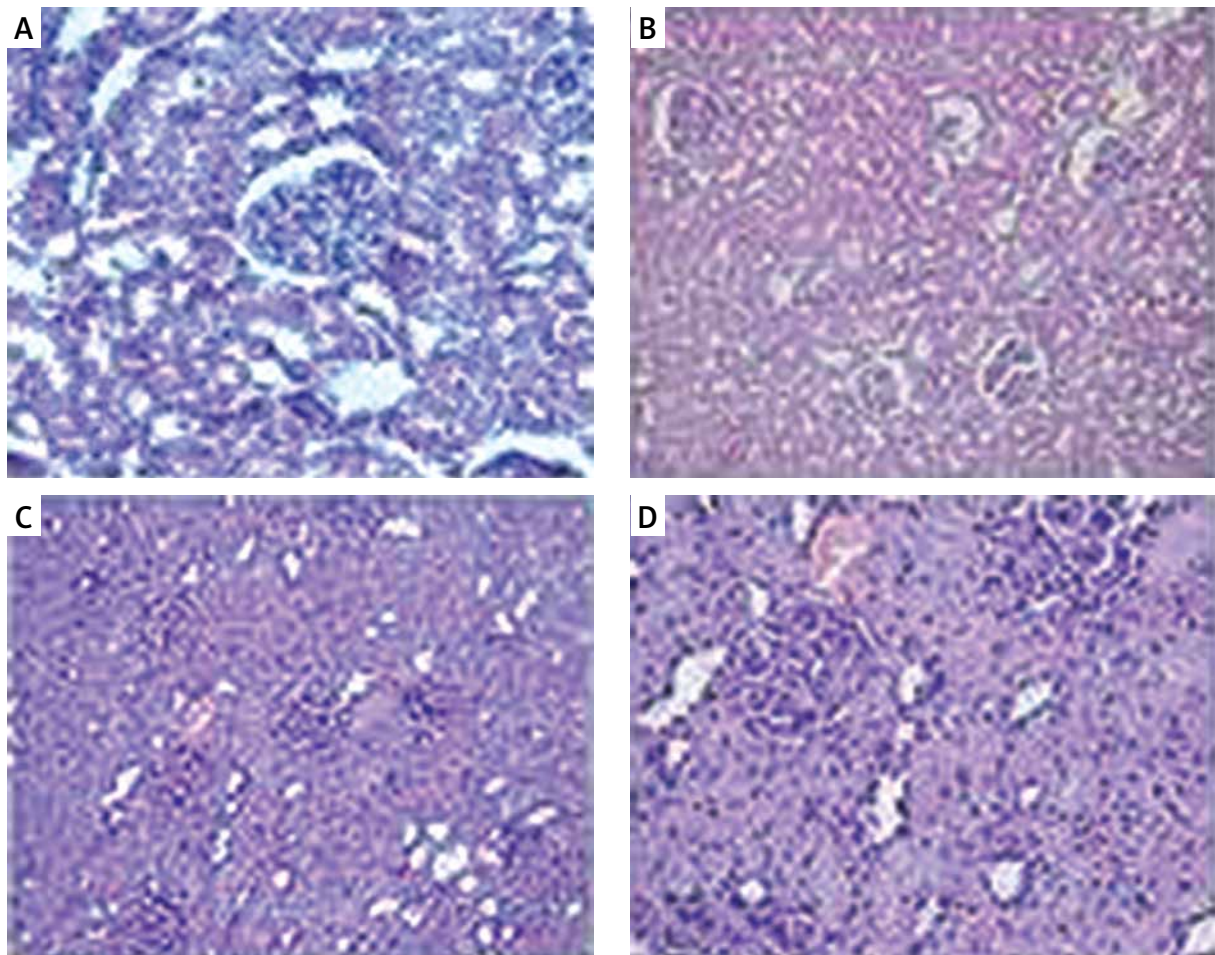




**Figure 3.** Graph of total scores of histopathological analysis of the kidneys by groups

We found the highest degree of renal injury in the 3 h PP group with a total renal injury score of 5 on histopathological evaluation and the lowest score in the control group (Figure 3 and Photo 1). Total scores were significantly different between the groups ( $p < 0.001$ ). The more prolonged the duration of PP was, the greater was the injury to the kidney.

In light of our findings, increases in NGAL and Cys C levels directly correlated with the duration of PP and IAP, which are therefore good biomarkers for diagnosing acute renal injury in its earliest phase. Plasmatic SCr level, contrary to what was expected, decreased, and it is not a good biomarker of early renal injury.



**Photo 1.** Histopathological features (light microscopy). **A** – control group: tubulointerstitial features in normal limits ( $1 \times 200$ ), **B** – 1 h pnp.: mild edema, mild congestion and glomerular lobulation ( $1 \times 200$ ), **C** – 3 h pnp.: congestion of glomeruli, edema of tubular epithelium, swelling of endothelial cells and vascular congestion ( $1 \times 200$ ), **D** – 3 h pnp.: congestion of glomeruli, edema of tubular epithelium, swelling of endothelial cells and vascular congestion ( $1 \times 400$ )



On histopathological analysis of the kidneys, we found that the degree of renal injury varied in proportion to the increase of these two biomarkers. Depending on the PP duration, IAP resulted in an increase of the degree of kidney injury and an increase in the levels of these two biomarkers.

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## Conflict of interest

The authors declare no conflict of interest.

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