Kidney Damage After Sepsis: A Long-term Follow-up. An Experimental Study.

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Title: Kidney damage after sepsis: a long-term follow-up. An experimental study.

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Abstract

Background: Acute kidney injury (AKI) in sepsis is a common event. This study aims to evaluate the long-term impact of sepsis on renal hemodynamics and morphology.

Methods: Wistar rats underwent sepsis and survivors (n = 24) were followed for up to six months, monitoring macro, regional and micro hemodynamics of the kidney, serum creatinine, and renal histology. The naive animals were used as the control group (n= 6), and sepsis was induced by *E. coli* e.v. inoculation. Surviving animals were monitored for up to six months. **Results:** Overall, the findings show that sepsis survivors have long-term hemodynamic and morphological compromise, as well as a progressive worsening of renal functional unit components over time. Even after six months of recovery from sepsis, severe renal hypoxia, chronic inflammation, evidence of increased vascular resistance, and renal fibrosis were observed in surviving animals. These alterations were present in animals with a healthy appearance and normal MAP. **Conclusion:** Those findings may represent a state of severely impaired physiology and be a contributing factor to the higher susceptibility to renal failure in the face of a new infectious challenge or to other pathological stimuli in the post-sepsis periods.

Keywords: post-sepsis, sepsis survivor, chronic kidney disease, renal hypoperfusion, microcirculatory disfunction, long-term kidney dysfunction, renal disease.

Introduction

Sepsis is a multifactorial inflammatory disease associated with organ failure and death. [1]. Currently, while mortality from sepsis in hospitals has been decreasing, mortality post sepsis remains at elevated levels [2-6]. The post-sepsis period's pathophysiological mechanisms are not clarified. [6-8].

The main pathological hemodynamic characteristics in patients with sepsis and septic shock include distributive shock, myocardial depression, altered microvascular flow, and diffuse dysfunction of the endothelial barrier, which results in microvascular leakage, edema of tissues and organs. [8-11]. Microcirculatory dysfunction can induce an imbalance between systemic oxygen supply and demand, leading to global tissue hypoxia and shock. [11-12].

Acute kidney injury (AKI) remains a frequent event in severe sepsis due to systemic hemodynamic disorders associated with an exacerbated systemic inflammatory state. [13-14]. Up to 60% of patients with sepsis have AKI and patients with AKI associated with sepsis have significantly increased mortality compared to those with AKI of another etiology. [15].

Microcirculation dysfunction in sepsis may cause the development of AKI, because of the decrease in the supply of oxygen, leading to areas of hypoxia/ischemia, and a decreased nutrient supply. [16]. Localized hypoxia can generate changes in metabolic, inflammation, and tissue damage.[17]. Tubular injuries, apoptosis and necrosis have been associated with AKI in sepsis. [18-19].

Considering the complexity of the pathophysiological mechanisms of kidney changes in sepsis, the understanding of the consequences of the disease in survivors may bring new knowledge for the intensive care of patients with sepsis. This study was designed to assess whether the kidney injury due to sepsis recovers or can persist, such as residual damage, representing a condition that can limit the ability to have an adequate renal physiological response in the case of reinfection in survivors.

Results

MAP

The MAP remained within the normal range (106 ± 9 mmHg) in all groups at all time periods of the study.

Renal blood flow

Despite maintaining normal MAP levels, renal artery and vein flow were significantly reduced in the very acute phase of sepsis (6h) compared to the Naive group (n=6) data. (Figure 1).

Figure 1,

Surprisingly, in the post-sepsis periods, the surviving animals (n=24) persisted with a significant hypo flow even up to six months, despite presenting a healthy clinical aspect with good mobility and with weight gain.

Although there was a trend in the recovery in the first month, a drop of around 50% was observed by the third month, and there was a partial recovery in six months (30-40% decrease). The percentage change shows how big the change is relative to the initial value (control group). The percentage was calculated using the following equation: $[X \text{ (final)} - X \text{ (initial)} / X \text{ (initial)} * 100 \text{ percent}]$ (Figure 2).

Figure 2.

A
complete

restoration of kidney blood flow was not achieved, both in the artery and in the renal vein, demonstrating that a single episode of sepsis causes significant long-term blood flow alterations in sepsis survival.

Cortical tissue perfusion unit (Laser Doppler)

Similarly, renal cortex tissue perfusion showed a matched hypoperfusion pattern (Figure 3), suggesting that kidney tissue hemodynamics are directly influenced by the regional flow, even in the absence of macrohemodynamic changes (MAP).

Figure 3.

In all post-sepsis phases, cortical tissue perfusion was significantly reduced. Within 30 days, there was a partial recovery, followed by a gradual decline for up to six months. When compared to the naive group, hypoperfusion was considerable at the end of six months (baseline). This pattern demonstrates that the renal cortical blood flow is significantly reduced and does not return to its baseline.

Renal cortex microcirculation hemodynamics (SDF)

Videomicroscopy of the renal cortex microcirculation revealed significantly altered cortical tissue structures as well as microcirculatory flow. In the very acute phase of sepsis (6 hours), there were observed generalized alterations in the architecture of the renal tubules, with a reduction in the luminal space and edema of the epithelial cells. The peritubular microvessels, which had venous characteristics, were congested or compressed as the tubular wall grew larger. These patterns of structural

disarrangement produce the perception of a heterogeneous look due to tubular-vascular zones that connect the preserved and compromised portions to variable degrees. (Figure 4-SDF). The hemodynamics of microcirculation in survivors improved gradually but only partially over time. However, even after six months, there was no complete recovery. This could be one of the factors driving chronic renal hypoxia/ischemia due to decreased blood flow in the renal artery and vein.

Histological findings

Naive: Homogeneous kidney structure distribution, with a normal aspect of the epithelial cells of convoluted tubules, preserved luminal space, peritubular microvessels with normal distribution, and with preserved intravascular space. The glomeruli had preserved urinary space and capillaries. (Figure 4-HE).

6 hours: The renal parenchyma is heterogeneous, with considerable congestion of venous and peritubular microvessels. The tubular lumen is significantly reduced by epithelial cell edema, and surrounding peritubular microvessels are compressed or collapsed. There is capillary congestion and a modest reduction in the urine space in the glomeruli. The data point to a broad picture of renal tubule structural degeneration and microcirculatory dysfunction. (Figure 4-HE).

30 days: Diffuse persistence of congestion of microvessels and venules, visible reduction of tissue and parenchymal spaces, severe edema of the tubular epithelium, and tubular lumen reduction. A hyaline substance can be found inside some convoluted tubules. There was a significant worsening of blood congestion in the capillaries of the glomeruli, as well as a significant decrease in their urinary space. In addition, infiltration of mononuclear leukocytes was observed focally. (Figure 4-HE). These findings were consistent with the evolution of renal derangement and the persistence of renal damage associated with decreased venous return and glomerular influx. It's worth noting that these findings were found in a one-month sepsis survivor who appeared to be in good health clinically. The presence of mononuclear infiltrates in the renal parenchyma, on the other hand, suggested persistent inflammatory processes.

90 days: There was a partial improvement in venous congestion and glomerular capillaries, but with the persistence of congestion in peritubular microvessels. There was still a reduction in the luminal space of the convoluted tubules associated with edema of the epithelial cells, and the presence of a hyaline substance in the tubular lumen. However, a partial recovery of tubules with a visible lumen was observed. The majority of peritubular microvessels, on the other hand, remained congested or compressed due to tubule epithelial cell edema. The peritubular space remained infiltrated with localized mononuclear cells. The urine space in the glomerulus was

slightly increased. (Figure 4-HE). The findings revealed a partial recovery phase after three months after sepsis survival, while there was still a broad change in the kidney's structural components.

180 days: Even after 6 months of survival, the global histological appearance showed the persistence of edema of the epithelial cells of the convoluted tubules, surprisingly more pronounced than at 3 months. Congestion of the large tortuous venules still persisted, with the hemosiderin inside. The continued quantitative loss of peritubular microvessels suggests that microcirculatory dysfunction has not recovered. Arterioles with thicker walls (dotted circle) also indicated an active atherosclerosis process. Most glomeruli narrow the urinary space, and some even thicken the glomerular capsule. In the capillaries of the glomeruli, the presence of an amorphous substance was observed between the loops, indicating collagen deposition. The increased deposition of amorphous substances seen between the capillary loops of the glomeruli was a suggestive sign of a progressive remodeling process. The findings of sepsis survivors after 6 months demonstrate that there is a process of ongoing renal injury and remodeling, which is consistent with an increase in vascular resistance. In addition, the tubular injury's slow healing and prolonged hemodynamic dysfunction all pointed to chronic kidney disease with fibrosis. (Figure 4-HE).

Owing to these histological findings, we next evaluated the collagen type.

Figure 4.

Collagen findings

The use of Picrosirius red staining on the histology slides revealed that changes occurred over a one-month period, with Type III (green) collagen predominating over Type I (red) collagen in the walls of glomeruli and tubules. There was a considerable prevalence of Type I at three months, which increased even more in the sixth months, particularly in the intercapillary spaces of the glomeruli, Bowman's capsule, and the convoluted tubule walls near to the glomeruli. Furthermore, there was a statistically significant deposition of collagen Type I in the thickened arteriole wall, as well as in certain veins and the renal matrix between the tubules, corroborating the arteriosclerosis process described by the histology. These findings strongly suggest the progression to multifocal renal fibrosis in the first six months after sepsis. (Figure 4- Picrosirius and Figure 5). The advancement of multifocal renal fibrosis in the first six months after sepsis is clearly suggested by this data. This means limited kidney structures to exert adequate kidney function in the post-sepsis period, and its vulnerability to a new infectious challenge or to other pathological stimuli. This indicates that in the post-sepsis period, renal structures are limited in their ability to exert proper

kidney function, making them vulnerable to infectious challenges or to other pathological stimuli.

Figure 5.

Discussion

AKI is a common complication of severe sepsis and is linked to systemic hemodynamic disturbances and an exacerbation of the host's systemic inflammatory state. [20]. The long-term renal effects of sepsis on survivors, on the other hand, are still unknown. [13,15]. Furthermore, it is unknown how much renal impairment caused by sepsis plays a role in hospital readmission or possibly death.

Hydroelectric homeostasis, blood volume management, blood pressure, pH balance, and plasma osmolarity are some of the kidney's physiological processes that help to repair the hemodynamic imbalance caused by critical illnesses. Injuries observed in AKI during sepsis include ischemia-reperfusion injury in the glomerulus, inflammation of sections of the nephron, oxidative stress, tubular injury mediated by cytokines and chemokines, and tubular and mesenchymal apoptosis. [16-17].

According to our findings, sepsis caused significant changes in the distribution of microcirculatory blood flow and severe morphological changes in the renal tissues by promoting microcirculatory dysfunction. The edema of tubule epithelial cells, which caused compression of their lumen and peritubular microvessels, resulting in microvascular congestion and restricted blood flow, was one of the most noticeable changes. When this occurs in a widespread manner, it creates congestion not just in the renal parenchyma's small and medium peritubular microvessels, but also in the glomerular capillaries of high blood flow. These effects were apparent from the acute phase of sepsis until the completion of a six-month recovery period.

Renal fibrosis and maladaptive repair may be associated to a condition of renal hypoxia/ischemia, an increase in renal vascular resistance, and the emergence of chronic pathogenic effects.

Several pathophysiological events occur simultaneously and in sequence during AKI, including microcirculatory failure, endothelial cell dysfunction, inflammation, recruitment of various leukocytes and cytokines, tubular cell damage, and tubular and renal venous congestion. [21-22]. Fibrosis is a feature of maladaptive repair, according to a crucial relationship between injury, improper repair, and the development of fibrosis. [23].

Pericyte to myofibroblast transition, endothelial failure, chronic inflammatory infiltrates, renin–angiotensin system activation, mitochondrial dysfunction, and epigenetic modifications are all pathological changes that contribute to renal fibrosis as a result of the maladaptive process. [24]. Other findings have highlighted the role of the proximal tubule and proximal tubular epithelial cell, a specialized tubular segment just adjacent to the glomerulus, as not only the target of injury but also an important mediator of renal fibrosis progression. [25].

Our findings regarding collagen repair also show that the fibrosis process is more evident in glomeruli, capillaries, and Bowman's capsule, and in the adjacent tubules. The growing prevalence of Type-I collagen over Type-III collagen and the progressive thickness of Type-I fibers, notably in the three and six post-sepsis periods, indicated the advancement of renal fibrosis, and the increase in renal vascular resistance.

These findings support the hypothesis that the first six months after sepsis represent a critical period for a new infectious insult. During this period, damage to the functioning kidney units provides a favorable environment for the disease. A study of patients aged 50 years or older than sepsis survivors demonstrated that they were 2.5 times more likely to be readmitted to the hospital for AKI within 90 days than patients with compatible comorbidity without sepsis. [26].

Furthermore, a significant and long-lasting reduction in renal artery and vein blood flow, in conjunction with microvascular dysfunction and tissue hypoperfusion, shows that the kidneys are continuously exposed to the hypoxia/ischemia factor in post-sepsis periods.

Sepsis-induced AKI, according to Gomez et al., is the first clinical and biochemical expression of adaptive tubular cell response to a detrimental inflammatory danger signal [17]. They proposed that the interplay of inflammation and microvascular

malfunction defines and amplifies this signal, and that tubular cell mitochondria orchestrate metabolic dysregulation in response.

In conclusion, a single sepsis episode was able to trigger a cascade of changes that can impact renal physiology over an extended period of time, raising the question of whether this can happen in other organs commonly affected by sepsis. Certainly, the combined dysfunction of the damaged organs can impair the defense mechanisms' response capacity to prevent harmful outcomes.

To better comprehend this idea, we are looking into other organs that are involved in sepsis. Although experimental animal studies may not fully replicate the etiopathogenesis in human sepsis survivors' illnesses, the ethical concerns of invasive procedures in humans can be solved in experimental investigations, allowing new theories to contribute to post-sepsis disease understanding.

Methods

Experimental groups

Female Wistar-EPM rats, weighing on average 250 g, were obtained from the University Animal Colony (CEDEME-UNIFESP). The animals were allocated to plastic cages, given free access to a standard diet and water ad libitum in environmentally controlled conditions (temperature, 20–22 C°; relative humidity 40-60% and lighting 12 h light/12 h dark cycle). A total of 30 animals were randomized into the following four groups after sepsis induction (S8 – injection into the jugular vein of *E. coli*, 2 mL of 10⁸ CFU/ mL). S8T6h; S8T30d; S8T90d; S8T120d; S8T180d groups - animals were monitored after 6 hours, 30, 90, and 180 days of sepsis induction, respectively (n=6/period). Naïve Group (N) - animals injected with 2 mL of saline were used as a control (n=6). The study was approved by the Federal University of São Paulo - UNIFESP Ethical Committee (CEP 159908) and all authors declare that all experiments were performed in accordance with the ARRIVE guidelines.

All the methods were performed in accordance with the relevant guidelines and regulations.

Anesthesia and Surgical procedures

General anesthesia was induced by intramuscular injection of 0.1 mL/ 100g (body weight) of Ketamine and Hydroxychloral (4:1). After identification, isolation, and cannulation of the jugular vein with a catheter, sepsis was induced by an infusion of *Escherichia coli* R6 [27], 2 mL of 10⁸ CFU/ mL (Dose Lethal - DL=60% in 27 hours). After 6 hours, 30, 90, and 180 days, the surviving animals were submitted to macro, regional and microcirculation monitoring *in vivo* and the kidneys were collected for histological assay. All surgical procedures were carried out using aseptic and antiseptic

procedures. After experiments, the euthanasia of the animals was performed by the section of the aorta under general anesthesia.

Macrocirculation monitoring

Under general anesthesia, the animals were trichotomized in the abdominal region, immobilized on a heated surgical board (37° C) in the supine position. For the evaluation of mean arterial pressure (MAP), a small skin incision was made in the inguinal region, followed by the dissection and isolation of the femoral artery. Following the opening of the femoral artery, a catheter was inserted and fixed, and connected to a pressure transducer. For post-sepsis monitoring, rats were anesthetized with isoflurane 3% via inhalation and maintained throughout the *in vivo* monitoring with a 1.5mL/minute flow of oxygen.

Renal artery and vein blood flow.

The monitoring of blood flow of the renal artery and vein was realized by midline abdominal incision, isolation of renal vessels and placing a perivascular 0.7mm probe of the Transonic System Inc. TS420 connected to the flowmeter, under general anesthesia.

Kidney cortical area tissue perfusion

The kidney cortical tissue perfusion was measured by the BLF 21 Laser Doppler flowmeter, under general anesthesia. Three measurements per organ/per animal were obtained, and the results were expressed by the average of the tissue perfusion unit (TPU) readings.

Kidney microcirculation monitoring

The microcirculation of the kidney cortical area of all groups was obtained at 6h, 30,90, and 180 days after sepsis induction, by Sidestream Dark Field Imaging (SDF) videomicroscopy (MicroScan, MicroVision Medical Inc., Amsterdam, The Netherlands) [28]. Five measurements were performed per organ per animal. The results were compared between groups. All procedures were carried out while under general anesthesia.

Histological analysis

At the end of all the monitoring procedures, the kidneys were collected for a histological assay. The kidney samples were divided into quarters and fixed in a glutaraldehyde solution (2.5%) in cacodylate sodium buffer 0.1 M, pH 7.4. After a serial dehydration process in a solution of ethanol from 50 to 100% per 10 minutes, the samples were kept for about 12 hours in absolute ethanol and histoiresin (Thecnovit 7100 - 1:1). The samples were immersed in pure resin for a period of 4 hours and subsequently embedded in resin and polymerized in appropriate plastic molds. [29] The histologic

sections of 3 μm thickness were stained by hematoxylin/eosin (HE). The histological analyses were performed under a light microscope, Carl-Zeiss Axio Scope.

Collagen analysis

To measure the course of kidney damage, the collagen analysis was solely compared with acute sepsis and post-sepsis recovery phases. Kidney fragments were fixed with 4% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), resin embedded (Technovit Kit 7100), and cut into 4 μm -thick slices. The tissue slices were stained with Picrosirius Red and were examined under polarized light on a Carl-Zeiss Axio Scope Microscope. All images were acquired following identical camera settings, including white balance, gain, and exposure. Images were taken at 40x magnification and the intensity of red (Type III) and green (Type I) collagen fiber staining was analyzed with the "rectangle profile" tool of the Zen software, utilizing similar methods described previously by Souza et al. [30-31].

Creatinine values

During the laparotomy, a venous blood sample was collected from the vena cava for analysis in a rapid test using a creatinine kit by I-STAT System (ABBOTT).

Statistical analysis

The nonparametric statistical test Kruskal-Wallis with Dunn correction for multiple comparisons [24] was used to quantify collagen Types I and III, as well as blood flow between study periods. GraphPad Prism 5.0 was used to perform collagen statistical analyses. Differences were considered statistically significant at a p-value ≤ 0.05 .

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contribution

AMAL: conception, design, methodology, formal analysis, acquisition of data, writing and review.

GCO: Acquisition of data.

RBS: Histology, histological analysis.

LLC: Histology, histological analysis.

IHJK: conceptualization, design, analysis, writing, supervision, revision of the manuscript and funding acquisition.

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Disclosures

No conflicting relationship exists for any author.

Figure Legends

Figure 1. Renal artery and vein blood flow at the early acute phase of sepsis (6 hours) and at all post-sepsis periods (30 to 180 days). Data were expressed in average of the bloodflow (mL/minute). The significant difference between groups were represented as: *Significant difference to naive group; ⁰ Significant difference between 6h and other groups. ($p \leq 0.05$).

Figure 2. Representation of the percentage (%) decline in renal artery and vein flow during sepsis periods relative to the naive group's baseline (baseline = 0 %). The percentage was calculated using the following equation: $[X \text{ (final)} - X \text{ (initial)} / X \text{ (initial)} * 100 \text{ percent}]$.

Figure 3. Kidney cortical area blood perfusion at the early acute phase of sepsis (6 hours) and at all post-sepsis periods (30, 90 and 180 days). Data were expressed in the average of the tissue perfusion unit (TPU). The significant difference between groups were

represented as: (*) Significant difference to naive group; (⁰) Significant difference between 6h and 30d groups; (#) Significant difference between 30d and 180d groups. ($p \leq 0.05$).

Figure 4-SDF. Kidney cortex microcirculation images: Naive: The intact rat kidney showed homogeneous-looking renal cortex with convoluted tubules (white arrow) interspersed with peritubular venules (black arrow); 6h: generalized appearance of tubular edema (white dotted arrows) and some congested peritubular venules (black dotted arrow); 30 days: partial improvement of tubular edema (white dotted arrow), less compression of the venules, but some venules with congestion still remained (black dotted arrow); 90 days: Similar to 30 day's findings, but with minor tubular edema (white dotted arrow) and with the glomerular capillaries congestion (black arrowhead); 180 days: Most tubules with edema (white arrow) and general dilation of peritubular venules (black arrow).

Figure 4-HE. The panel shows the histological images of the kidney over 6 months after the sepsis challenge compared to Naive. HE staining. Histological findings showed that there is a process of continuous renal damage, involving the glomerular capillaries (yellow arrow); glomerulus urinary space (yellow dotted arrow); amorphous substance in glomerulus (red arrowhead); thickening of the glomerular capsule (red arrow); peritubular microvessels (black arrow); leukocyte infiltration (blue arrow); microvessels congestion (white arrow); hemosiderin deposition (black arrow) and arteriole wall thickening (dotted circle).

Figure 4-Picrosirius. Images of collagen type-I (red) and type-III (green) expression by polarized light in post-sepsis periods, and their statistical differences. Picrosirius Red staining. (*) statistical difference between periods. ($p \leq 0.05$).

Figure 5. Measurements of the intensity of green (collagen type-III) and red (collagen type-I) in histology slides stained with Picrosirius Red under polarized light. (***) = statistical difference between the sepsis phases. ($p \leq 0.05$). There observed an increasing and considerable deposition of collagen type-I in the glomeruli and neighboring peritubular tubules during the post-sepsis periods, especially between 3 and 6 months.