HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

الجمعية السعودية لعلوم الحياة
SAUDI BIOLOGICAL SOCIETY

Original article

Improvement of renal functions in mice with septic acute kidney injury using secretome of mesenchymal stem cells

Arifin Arifin [a,b], Bambang Purwanto [a,b], Dono Indarto [a,c,*], Brian Wasita [a,d], Tatar Sumanjar [a,b], Eti Poncorini Pamungkasari [a,e], Soetrisno Soetrisno [a,f]

[a] Doctoral Program of Medical Sciences, Faculty of Medicine, Universitas Sebelas Maret, Jl. Ir Sutami No 36A, Kentingan, Jebres, Surakarta 57126, Indonesia
[b] Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret/General Hospital Dr. Moewardi, Jl. Kolonel Sutarto No. 132, Jebres, Surakarta 57126, Indonesia
[c] Department of Physiology and Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Jl. Ir Sutami No 36A, Kentingan, Jebres, Surakarta 57126, Indonesia
[d] Department of Anatomic Pathology, Faculty of Medicine, Universitas Sebelas Maret, Jl. Ir Sutami No 36A, Kentingan, Jebres, Surakarta 57126, Indonesia
[e] Department of Public Health, Faculty of Medicine, Universitas Sebelas Maret, Jl. Ir Sutami No 36A, Kentingan, Jebres, Surakarta 57126, Indonesia
[f] Department of Obstetrics and Gynaecology, Faculty of Medicine, Universitas Sebelas Maret, Jl. Ir Sutami No 36A, Kentingan, Jebres, Surakarta 57126, Indonesia

ARTICLE INFO

Keywords:
Secretome-MSCs
Inflammation
Tissue damage
Acute kidney injury
Sepsis

ABSTRACT

Background: A potentially fatal complication of sepsis is septic acute kidney injury. Stem cell therapy is a potential new method of treating sepsis and has been applied to treat some human diseases.
Objectives: This study investigated the effects of secretome-MSCs on NGAL, CRP, NF-κB, and MMP-9 proteins, and histopathology in mice with septic AKI.
Methods: A post-test-only group design was conducted in 30 Balb/C male mice, which were randomly assigned to five groups: the control group was intraperitoneally injected with 0.5 ml of 0.9 % NaCl, the septic AKI, and the treatment groups (T1, T2, and T3) were intraperitoneally injected with 0.5 ml of 0.9 % NaCl and 0.3 mg/kg BW LPS single dose for three days. Three-day treatments of 150, 300, and 600 μl secretome-MSCs were administered intraperitoneally into the treatment groups. Furthermore, kidney and blood samples were collected for biochemical and histopathological analyses.
Results: The T1, T2, and T3 groups had lower expression of NF-κB and MMP-9 and significantly lower CRP and NGAL levels than that of septic AKI group. T1 (1.21 ± 0.19), T2 (0.75 ± 0.22), and T3 (0.38 ± 0.14) groups demonstrated lower average scores for inflammation, necrosis, hemorrhage, and degeneration compared to septic AKI group (2.17 ± 0.13).
Conclusions: Administration of 600 μl/20 g BW secretome-MSCs suppresses NF-κB and MMP-9 expression and reduces CRP and NGAL levels. Meanwhile, the 150 and 300 μl/20 g BW doses also indicated a greater improvement in renal tissue damage of mice with septic AKI.

Abbreviations: AKI, Acute kidney injury; Ang-II, Angiotensin-II; ANOVA, Analysis of Variance; BW, Body weight; COVID-19, Corona virus disease-19; CRP, C-Reactive Protein; ELISA, Enzyme-linked immunosorbent assay; ENOS, Endothelial nitric oxide synthase; ERK, Extracellular signal-regulated protein kinase; ET-1, Endothelin-1; g, gram; HGF, hepatocyte growth factor; IgG, Immunoglobulin G; IL, Interleukin; LPS, Lipopolysaccharide; LSD, Least significant difference; miRNA, micro ribonucleic acid; MMPs, Matrix metalloproteinases; MMP-9, Matrix Metalloproteinase 9; MSCs, Mesenchymal Stem Cells; NADPH, Nicotinamide adenine dinucleotide phosphate; NF-κB, Nuclear Factor-Kappa B; ng/L, nanogram/litre; NGAL, Neutrophil Gelatinase-Associated Lipocalin; NO, Nitric oxide; NOS, Nitric oxide synthase; PI3K, Phosphatidylinositol 3-kinase; ROS, Reactive oxygen species; SD, Standard deviation; TNFα, Tumor necrosis factor alpha; U/L, Unit/litre; ul, microliter.

* Corresponding author at: Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia.
E-mail addresses: arifinkic@staff.uns.ac.id (A. Arifin), bambang_p48@staff.uns.ac.id (B. Purwanto), dono@staff.uns.ac.id (D. Indarto), brianwasita@staff.uns.ac.id (B. Wasita), tatar_solo@staff.uns.ac.id (T. Sumanjar), etiponco@staff.uns.ac.id (E.P. Pamungkasari), soetrisno@staff.uns.ac.id (S. Soetrisno).

https://doi.org/10.1016/j.sjbs.2024.103931
Received 30 March 2023; Received in revised form 23 December 2023; Accepted 11 January 2024
Available online 13 January 2024

1. Introduction

Sepsis is a severe medical condition that is characterized by life-threatening dysfunction of organs resulting from irregular responses of the host to an infection (Rhodes et al., 2017). This condition is becoming a major health problem around the world, with more than 30 million individuals affected, resulting in 6 million fatalities worldwide each year (Gyawali et al., 2019). Furthermore, sepsis can cause organ damage in several human bodies, including the brain, lungs, heart, liver, and kidney. Acute Kidney Injury (AKI) caused by infection (septic AKI) is a potentially fatal complication of sepsis (Peerapornratana et al., 2019).

Multiple inflammatory reactions in sepsis are regulated by specific cytokines (Steinhagen et al., 2020), in response to lipopolysaccharide (LPS), triggering the nuclear translocation and activation of NF-κB protein and producing several cytokines, including Tumor Necrosis Factor (TNFα), IL-1β, IL-6, IL-12, and IL-18 (van der Poll et al., 2017). Elevated IL-6 levels induce the hepatocytes to secrete CRP, reduce the endothelial nitric oxide synthase (eNOS) activity, and increase a vasoconstrictor of endothelin-1 (ET-1) levels (Savoia et al., 2011a). Together, those proteins increase Reactive Oxygen Species (ROS) generation, which turns in endothelial dysfunction and vascular inflammation (Savoia et al., 2011b).

Enhanced ROS and chronic inflammation of endothelial cells in the kidney can cause fluid and cell leakage, reduce organ perfusion, and lead to local ischemia and tubular damage (Bermejo-Martin et al., 2018). Additionally, the high and active expression of MMPs, specifically MMP-9, can contribute to the degradation of Extracellular Matrix (ECM) proteins such as collagen type IV, a critical component of the basal membrane in the kidney required for basal membrane remodeling during embryonic development (Zakiyanov et al., 2019). The endothelial cells in the kidney are also susceptible to necrosis, which produces NGAL protein, which serves as a self-defense mechanism and a growth biomarker for tubular cells' differentiation (Dai et al., 2015; Wajda et al., 2019).

Renal replacement therapy is commonly used to treat septic AKI patients, but it has a high cost and low efficacy (Siddiqui et al., 2020). In recent years, stem cell therapy has been a potential new method of treating sepsis (Martínez et al., 2017). For instance, MSCs have been applied to treat some human diseases but they must be intensively cultured to achieve the optimal number of cells for transplantation (Harrell et al., 2019). The secretion of secretome-MSCs containing cytokines, microRNA (miRNA), exosomes, and microvesicles has a better therapeutic effect (Madrigal et al., 2014), which is directly available for acute therapy with bioactive sources analogous (Harrell et al., 2019). A previous study reported that administration of cytokines of secretome-MSCs could improve endothelial glycocalyx and phagocytic function of neutrophils and monocytes, and suppress NF-κB expression (Mizuno and Nakamura, 2012). Therefore, the aim of this study was to investigate the effects of secretome-MSCs on the NF-κB, MMP-9, CRP, and NGAL proteins and kidney tissue damage in the septic AKI mice model.

2. Materials and methods

The secretome-MSCs (CM-UCMSCD01) used in this study were obtained from the Stem Cells and Cancer Institute of PT Bifarma Adiluhung Jakarta, Indonesia. These cells were derived from the conditioned media of umbilical cord MSCs. Furthermore, the secretome-MSCs contained some growth factors, including 275.47 pg/ml of Hepatocyte Growth Factor (HGF), 50–300 ng/ml of pro-collagen, 30–300 pg/ml of keratinocyte growth factor/Fibroblast Growth Factor (FGF) 7, 300–1500 pg/ml of basic FGF 2, 10–50 pg/ml of stromal cells-derived factor 1/C-X-C motif chemokine 12, and 10–50 pg/ml of vascular endothelial growth factor.

2.1. Development of sepsis mice model and secretome-MSCs experiment

The mice with septic AKI were the subjects in this investigation, using a post-test-only control group design. The sample size was determined using a formula from the Institutional Animal Care and Use Committee (Office of Laboratory Animal Welfare, 2002), and ethical approval was obtained from the Health Research Ethics Committee of Dr. Moewardi General Hospital with number 7686/VIII/HREC/2021.

A total of 30 male *Mus musculus* mice of Balb/C strain, weighed 20–30g, aged 3–4 months, and acquired from the Faculty of Veterinary Medicine, Gadjah Mada University, Jogjakarta province were used in this study. For sepsis development, we only used male mice because of several reasons. At first, proestrus female mice had a significantly increased survival rate compared to male mice with sepsis, which were generated using the cecal ligature and puncture technique. Secondly, female mice with sepsis using the endotoxic shock (ExSh) technique were more resistant than their counterparts. The next reason was progesterone-regulated immunological responses and protection against infectious diseases by reducing inflammatory cytokines, IL-6, and TNF-α, and restoring the antioxidant defense system (Angele et al., 2014). Early production of TGFβ1in the lungs of female mice with sepsis induced by the ExSh was associated with increased survival and reduced lung injury (Bojalil et al., 2023). Based on our initial study, the optimal dose for generating mice with sepsis was the administration of 0.3 mg/kg BW single dose of *Escherichia coli* LPS, which indicated the most severe organ damage and significant increases of inflammatory markers (Arifin et al., 2023).

Selected mice were randomly distributed into five groups: the control group was intraperitoneally injected with 0,5 ml of 0,9% NaCl. The septic AKI and the treatment groups (T1, T2, and T3) were intraperitoneally injected with 0.5 ml of 0.9 % NaCl and 0.3 mg/kg BW LPS (Sigma-Aldrich USA, Cat# L2630) single dose for three days, respectively. Subsequently, three days of treatment of 150, 300, and 600 μl secretome-MSCs were administered intraperitoneally into the treatment groups (T1–T3). All mice were given a standard mouse feed and freely accessed drinking water. At the end of the third day of treatment, all mice were sacrificed, and their kidney, blood, and urine samples were collected for further analysis.

2.2. Measurement of serum CRP and urine NGAL levels

The serum CRP and urine NGAL levels were determined using the ELISA assay kits purchased from Wuhan Fine Biotech, China (Cat# EM0061 and EM1232 respectively). The protocol for measuring these proteins was based on the manufacturer's protocols.

2.3. Immunohistochemical staining

All mice's kidneys were embedded using paraffin, which was cut with 0.5 mm thickness to get several slices. The slices of kidney tissues were attached on glass slides and were then incubated overnight with 1:1,200 dilution of NF-κB anti-mouse monoclonal antibody and 1:400 dilution of MMP-9 rabbit polyclonal antibody. The slides were incubated using an IgG rabbit secondary antibody linked to the horse radish peroxidase (HRP) for 30–45 min to recognize the interaction of antigen and antibody complexes. They were stained for 10 min with diaminobenzidine solution, which was the HRP substrate. Ultimately, the slides were counterstained for one min with a hematoxylin solution (Salah, 2020). Moreover, under a light microscope with 200× magnification, the stained slides with positive staining of NF-κB and MMP-9 were scored using a semi-quantitative assessment. The intensity of NF-κB and MMP-9 cytoplasmic staining was scored 0 % = 0, <25 % = 1, 26–50 % = 2, 51–75 % = 3, and >75 % = 4 (Fedchenko and Reifenrath, 2014; Liu et al., 2019; Renaldi et al., 2023).

2.4. Histopathological staining

The remaining paraffin-embedded kidney tissues were cut at 0.5 mm thickness and stained with a hematoxylin-eosin solution. The pathological processes in kidney tissues were assessed under a light microscope with 4- and 200-times magnification such as degeneration, necrosis, inflammation, and bleeding. The histological evaluation was performed by two independent pathologists, and the findings were classified as 0: none, 1: mild, 2: moderate, 3: strong, as well as 4: severe (Kiyonaga et al., 2020).

2.5. Statistical analysis

Numerical data were assessed for normality and homogeneity using the Shapiro-Wilk and Levene's tests, respectively. Collected data of CRP and NGAL levels were reported as mean ± standard deviation, whilst scoring data of NF-κB MMP-9 were reported as percentages. The mean differences of CRP and NGAL levels among mice groups were statistically analyzed using the one-way ANOVA and then followed by the least significant difference post hoc test. Comparison of NF-κB, MMP-9, and kidney histopathology were analyzed statistically using the Mann-Whitney test and followed by the Kruskal-Wallis test at a significance level < 0.05.

3. Results

Administration of LPS in male Balb/C mice induced acute inflammation, resulting in septic AKI. Fig. 1A-F showed that the intensity of NF-κB immunostaining decreased in all groups treated with three different doses of Secretome-MSCs. The staining intensity in Fig. 1C and E was similar to Fig. 1A but weaker than Fig. 1B and D. In Fig. 1F, the average of NF-κB positive expressing kidney cells in the T1 (1.83 ± 0.41), T2 (1.67 ± 1.03), and T3 groups (1.33 ± 0.52) was significantly lower than septic AKI (2.83 ± 0.75).

The MMP9 expression in mice's kidneys was evaluated to confirm their organ damage, as shown in Fig. 2. The intensity of the immunostaining decreased in all mice groups treated with Secretome-MSCs. In Fig. 2F, the average of MMP9 positive kidney cells in the T1–3 (1.83 ± 0.41, 1.50 ± 0.55, and 1.00 ± 0.00, respectively) was significantly lower than septic AKI (2.83 ± 0.75).

The high expression of NF-κB protein stimulated TNF-α and IL-6 secretions, resulting in the release of CRP proteins by the hepatocytes. Fig. 3 showed that the administration of secretome-MSCs significantly reduced CRP levels in a dose-dependent manner. The average CRP levels in the treatment T1–3 (5.79 ± 0.39, 4.94 ± 0.17, and 4.03 ± 0.41 U/L, respectively) were significantly lower than the septic AKI group (15.61 ± 0.60 U/L; $p < 0.01$). However, reduced CRP levels in all treatment groups were higher compared to the normal group at 2.87 ± 0.24 U/L (see Fig. 3).

Besides MMP9 expressions, all treatment mice groups had a reduction of NGAL levels. Fig. 4 showed that the administration of secretome-MSCs significantly reduced NGAL levels in a dose-dependent manner, and the average of NGAL levels in the treatment groups (T1 = 57.37 ± 1.95, T2 = 47.33 ± 3.02, and T3 = 29.07 ± 1.80 ng/L) was significantly lower than septic AKI at 142.30 ± 3.47 ng/L. However, reduced NGAL levels in all treatments remained high compared to the NGAL in the normal group at 24.42 ± 0.74 ng/L.

Histopathological staining was conducted to evaluate the mouse kidney's inflammation process and tissue damage. Fig. 5 indicated that secretome-MSCs administration reduced inflammation, necrosis,

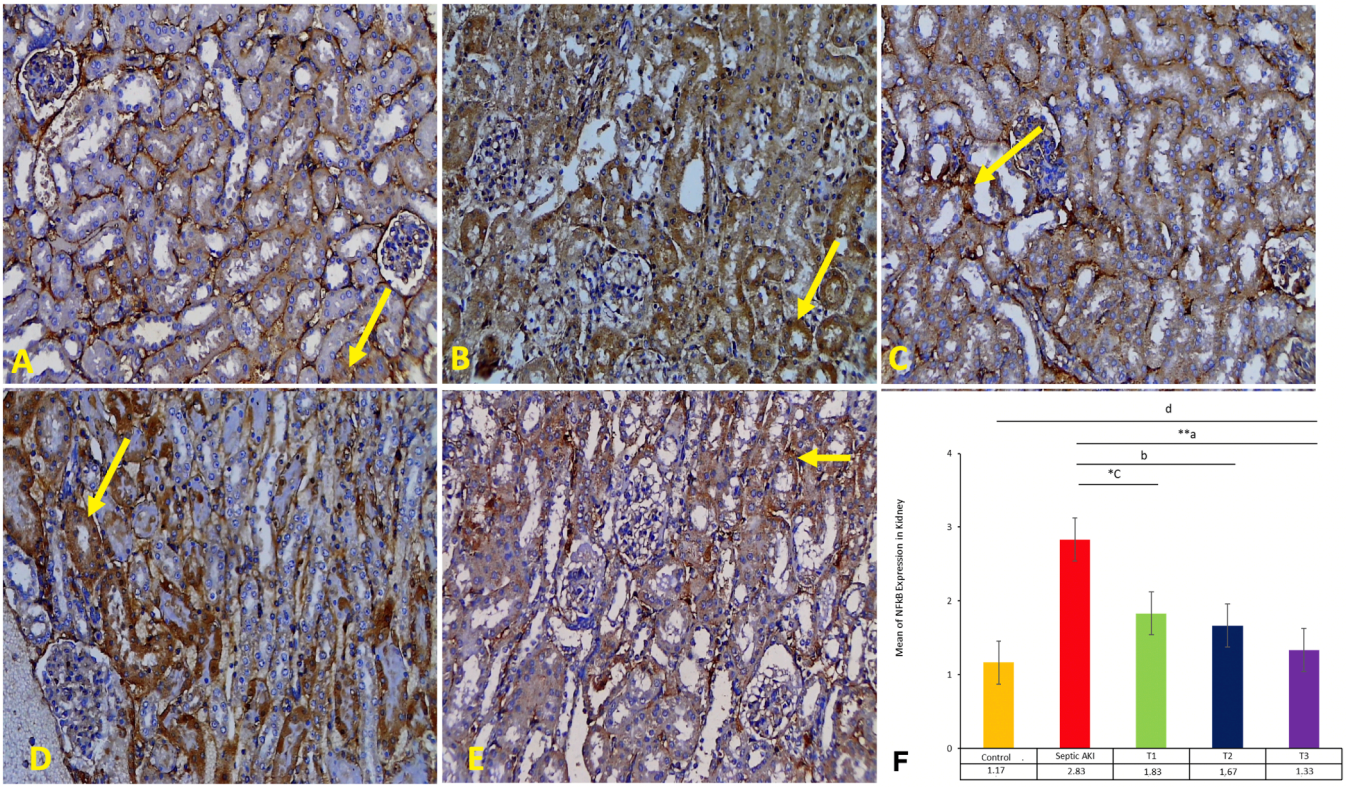

Fig. 1. Immunostaining of NF-κB expression in the mouse kidney tissues treated with and without secretome-MSCs. A-E indicated immunostaining in the normal, septic AKI, and treatment groups. The yellow arrow designated NF-κB expression in the proximal tubular epithelial cells of kidney tissue, and F indicated the scoring of kidney cells expressing NF-κB. Each bar represented 6 mice/group and * designated a significant difference at $\alpha < 0.05$ ** designated a significant difference at $\alpha < 0.01$. The immunostaining of all kidney tissues was assessed under a light microscope with 200× magnification. a,b, and c indicated significant differences among treatment groups compared to the septic AKI group, whilst d indicated significant differences between septic AKI and treatment groups compared to the control group.

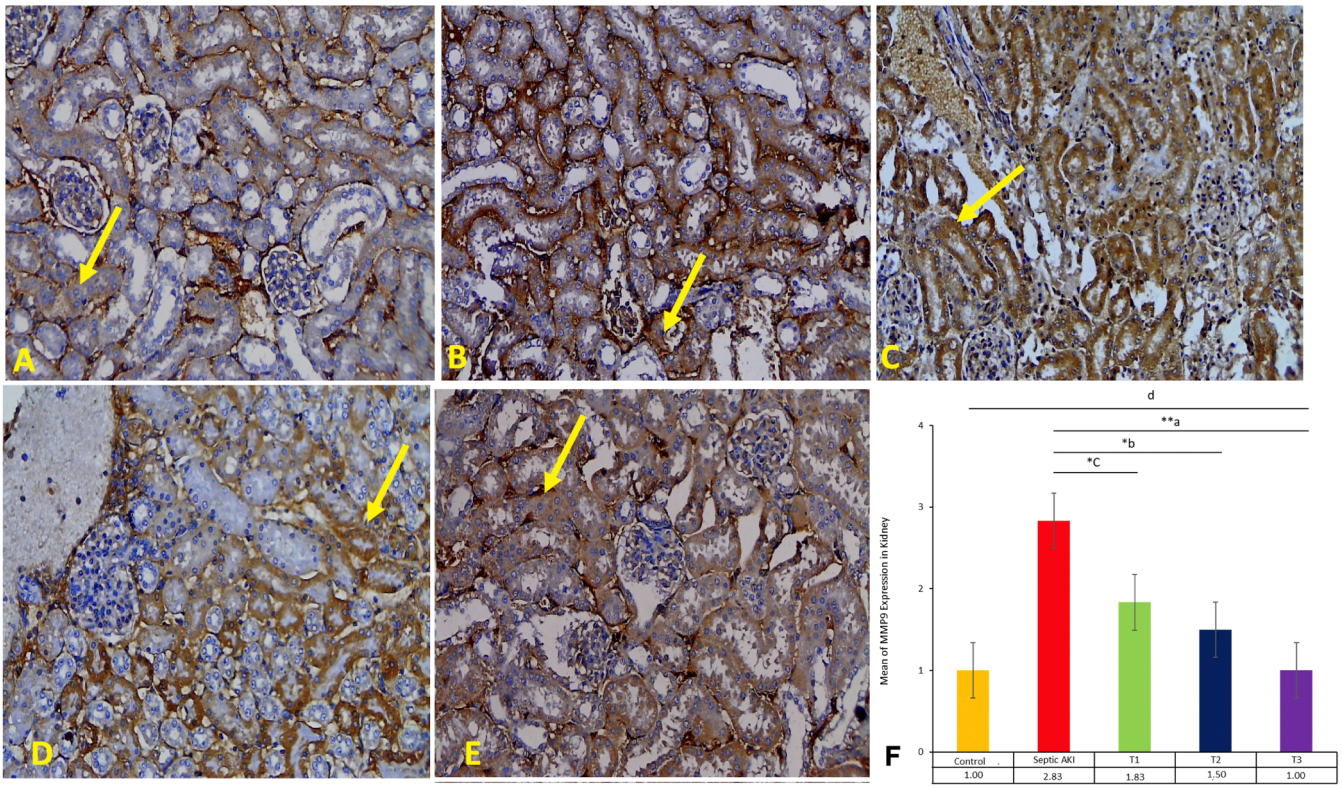

Fig. 2. Results of immunological staining of MMP9 expression in the mouse kidneys treated with and without secretome-MSCs. A-E indicated immunostaining in the normal, septic AKI, and treatment groups. The yellow arrow designated MMP9 expression in the proximal tubular epithelial cells of kidney tissues, and F indicated the scoring of kidney cells. Each bar represented 6 mice/group ± SD and * designated a significant difference at $\alpha < 0.05$ ** designated a significant difference at $\alpha < 0.01$. The immunostaining of all tissue sections was assessed under a light microscope with 200× magnification.

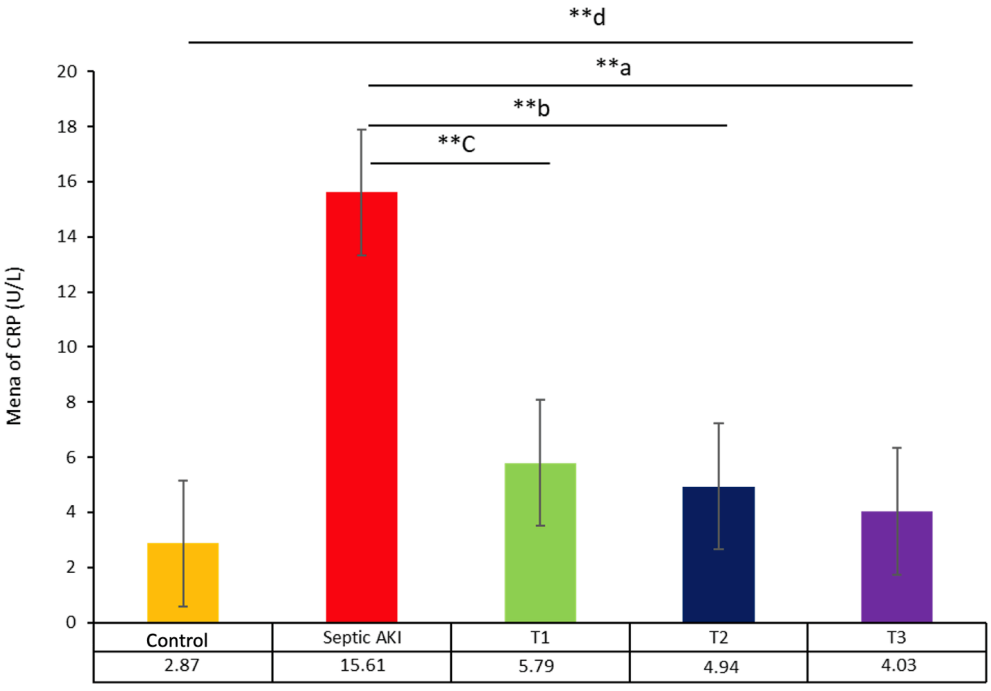

Fig. 3. The comparison of CRP levels between control and treatment groups with secretome-MSCs. Serum CRP levels among mice groups were measured using the ELISA method, and the average CRP levels were analyzed statistically using the one-way ANOVA and LSD post hoc tests. Each bar represented 6 mice/group and ** designated a significant difference at $\alpha < 0.01$.

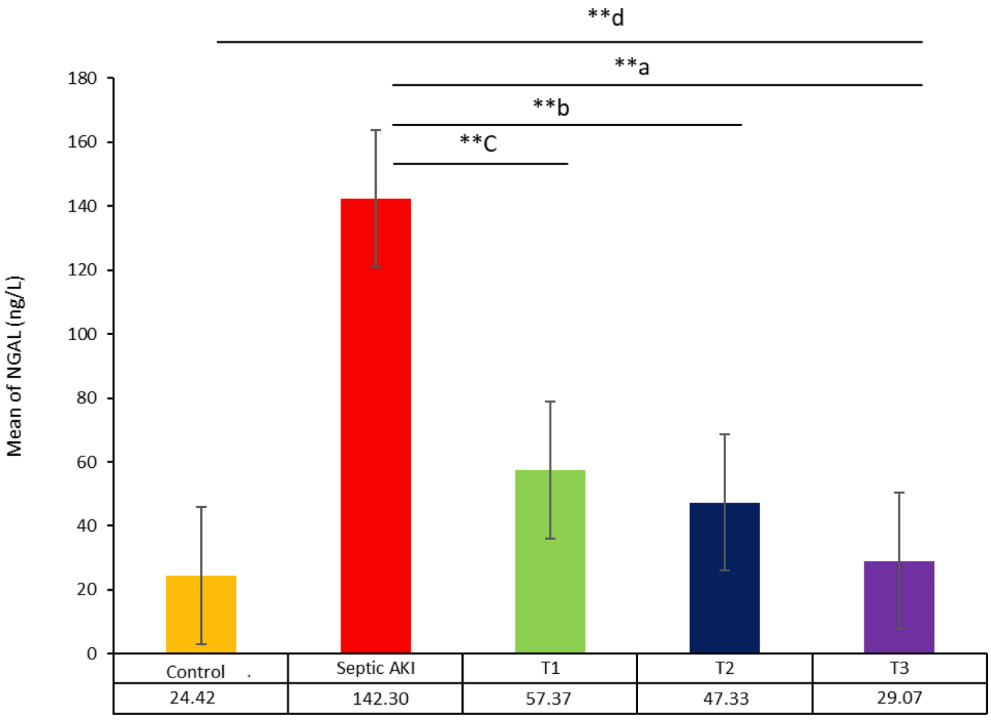

Fig. 4. The comparison of NGAL levels between control and treatment groups with secretome-MSCs. Urine NGAL levels among mice groups were measured using the ELISA method, and the average NGAL levels were analyzed statistically using the one-way ANOVA and the LSD post hoc tests. Each bar represented 6 mice/group and ** designated a significant difference at $\alpha < 0.01$.

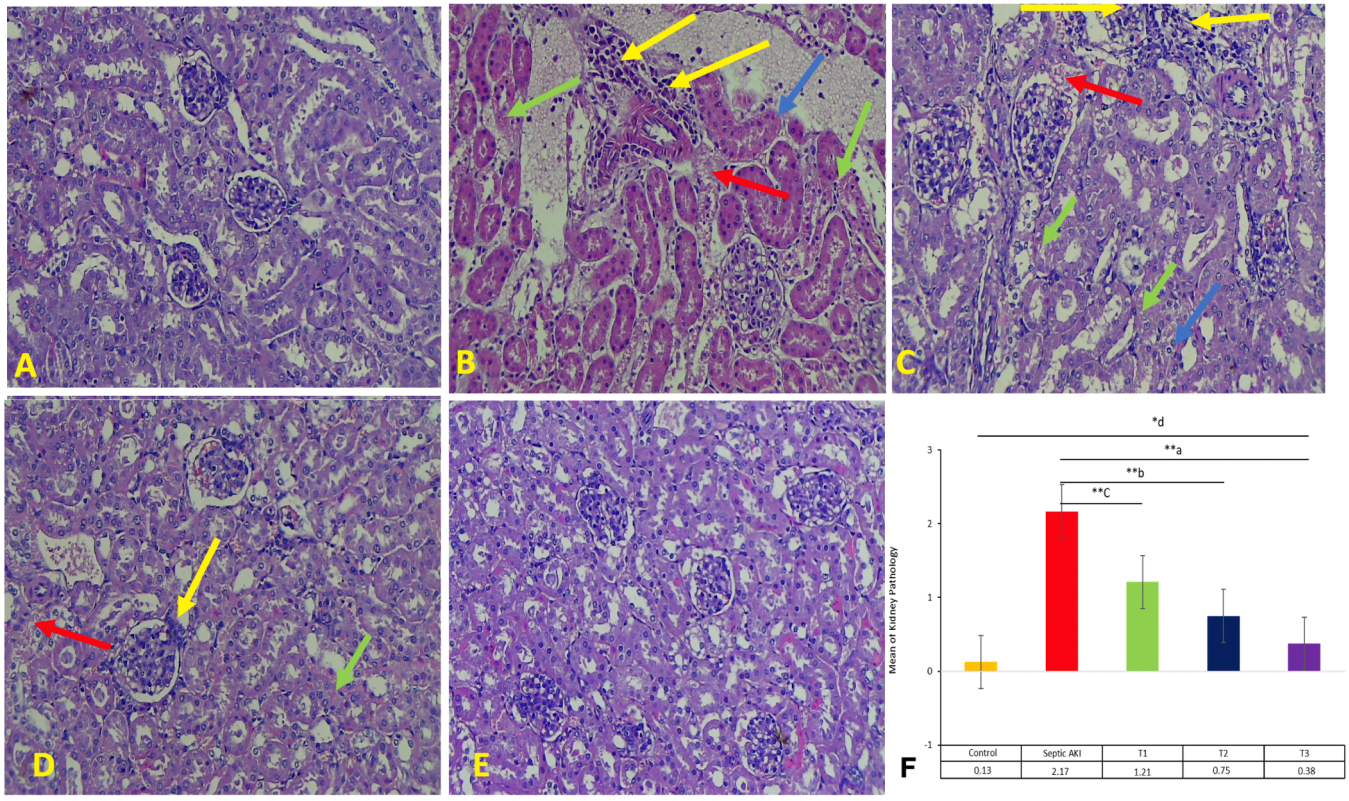

Fig. 5. Results of histopathological staining in the mouse kidneys treated with and without secretome-MSCs. A-E indicated histopathological in the normal, septic AKI and treatment (T1–3) groups. Yellow, green, red, and blue arrows represented inflammation, necrosis, hemorrhage, and degeneration in kidney tissues. Meanwhile, F indicated the scoring of the inflammation process in kidney tissues among groups. Each bar represented six mice/group and * designated a significant difference at $\alpha < 0.05$ ** designated a significant difference at $\alpha < 0.01$. The immunostaining of all tissue sections was assessed under a light Microscope with 200× magnification.

hemorrhage, and degeneration in kidney tissues with dependent doses. In Fig. 5 F, the average of inflammation, necrosis, hemorrhage, and degeneration scoring in the T1–3 (1.21 ± 0.19, 0.75 ± 0.22, and 0.38 ± 0.14, respectively) was significantly lower than septic AKI (2.17 ± 0.13) and normal groups (0.13 ± 0.14).

4. Discussion

This study showed that the administration of 600 μl secretome-MSCs can effectively inhibit NF-κB and MMP9 expression, reduce CRP and NGAL levels, and improve inflammation, necrosis, hemorrhage, and degeneration in kidney tissues with better responses than 150 and 300 μl. These results suggest that secretome-MSCs might become a promising AKI treatment. These findings corroborate the results of a previous study, where the treatment of MSCs can significantly reduce the expression of NF-κB in rat lung tissues induced by LPS (Su et al., 2019). Another study reported that reduced expression of NF-κB appeared in the lung tissues of mice sepsis model treated with MSCs. However, secretome-MSCs were used instead of MSCs, which could reduce the expression of NF-κB in the kidneys of the septic AKI mice model. The NF-κB transcription factor has a crucial role in regulating the inflammatory response and effectively modulates the control signals that determine proinflammatory gene transcription responses, including TNF, IL-1, and IL-6 (Pedrazza et al., 2017).

ROS production is also heightened, culminating in the inhibition of cellular bioenergetics, monocyte migration, tissue repair, and endothelial integrity with respect to the elevated expression of NF-κB. In addition to necrosis in kidney cells, sepsis also generates hepatocyte inflammation through the pro-inflammatory cytokine IL-6. This induces the synthesis of acute-phase proteins including CRP, antitrypsin α-1, fibrinogen, prothrombin, and haptoglobin (Woźnica et al., 2018). Furthermore, the CRP level in the T3 was 4.03 ± 0.41 U/L, close to the control group. These findings support a previous study conducted by Leng and co-workers that the administration of 1×10^6 MSCs/kgBW in patients with COVID-19 can decrease the serum level of CRP (Leng et al., 2020).

MMPs ECM is released by diverse inflammatory cells, predominantly neutrophils and macrophages. In sepsis, the MMP-9 has been implicated in acute lung injury, hepatic injury, vascular as well as cardiac dysfunction, and multiple organ failure (Niño et al., 2017). These findings indicated that the administration of secretome-MSCs significantly reduced MMP-9 expression dose-dependently. The reduced expression may lead to a decrease in type IV collagen degradation, a main kidney basal membrane component. Therefore, the remodeling of the kidney basal membrane could potentially improve tissue damage (Zakiyanov et al., 2019).

Furthermore, septic necrotic cells produce high NGAL levels as a biomarker of the growth and differentiation of renal tubular cells. The administration of 150, 300, and 600 μl secretome-MSCs reduced NGAL levels in mice with sepsis (T1 = 57.37 ± 1.95, T2 = 47.33 ± 3.02, and T3 = 29.07 ± 1.80 ng/L). These results are consistent with Yim et al, where the administration of 200 μl secretome-MSCs in rats with AKI also reduced NGAL levels (Yim et al., 2019). Therefore, NGAL levels could be utilized to indicate the AKI severity because of bacterial sepsis (Dai et al., 2015; Wajda et al., 2019; Wang et al., 2018).

In bacterial sepsis, evaluating tissue damage requires considering not only molecular but also cellular changes in AKI. The administration of secretome-MSCs significantly reduced histopathological scores of kidney tissues, such as necrosis, inflammation, hemorrhage, and degeneration in a dose-dependent manner. These findings strengthen a previous study conducted by Yim and co-workers that administering 200 μl secretome-MSCs can improve kidney tissue damage with low histopathological scores (Yim et al., 2019).

The findings suggest that secretome-MSCs can repair kidney tissue damage because of bacterial sepsis. In addition, the administration may alleviate kidney tissue damage by decreasing oxidative stress and fibrosis, inhibiting the expression of local and soluble inflammatory cytokines, increasing renal regulatory T lymphocytes, and promoting faster regeneration of epithelial kidney tubules (Bochon et al., 2019). The renoprotective effects may be related to high HGF and FGF levels in the method and material sections. The HGF plays vital roles in controlling inflammation by inhibiting the NF-κB pathway, directly reducing Glycogen synthase kinase 3β for NF-κB inactivation (Mizuno and Nakamura, 2012), and inducing NO production through NOS activation, resulting in the inhibition of E-selectin and intercellular adhesion molecules (Parajuli et al., 2012). Based on the results, it can reduce inflammation and kidney tissue injury in secretome-MSCs.

The potential nephroprotective effect of secretome-MSCs may be attributed to the presence of FGF in their secretome, which is involved in endothelial regeneration. The secretome-MSCs used in this study contained 300–1500 pg/ml FGF2, which has been shown to promote cell proliferation in various organs, including the liver, blood vessels, and skin. In fact, studies have shown that exogenous FGF2 can enhance the physiological function of different organs following ischemia–reperfusion injury through the activation of the PI3K and ERK1/2 pathways. However, these pathways were not assessed in mice with AKI in this study. Furthermore, the study's limitations include the lack of evidence regarding other growth factors in secretome-MSCs that may improve molecular and cellular levels in septic mice. The study design solely assessed the parameters after secretome-MSCs administration and did not compare the changes that occurred before the treatment.

5. Conclusions

The administration of 600 μl/20 g BW secretome-MSCs effectively inhibits NF-κB and MMP-9 expression and reduces CRP and NGAL levels, resulting in the improvement of kidney tissue damage in mice with septic AKI. However, further studies are required to fully evaluate the action mechanism of secretome-MSCs treatment in the PI3K and ERK pathways. The contribution of other growth factors in kidney tissue regeneration should also be investigated. Measurements of inflammatory and tissue damage parameters are expected to be evaluated before and after treatment with the secretome-MSCs.

Funding

The authors received no financial support for this article's study, authorship, and publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the research assistants and laboratory staff at the Faculty of Medicine, Universitas Sebelas Maret, for their help and support.

References

Angele, M.K., Pratschke, S., Hubbard, W.J., Chaudry, I.H., 2014. Gender differences in sepsis: cardiovascular and immunological aspects. Virulence 5, 12. https://doi.org/10.4161/viru.26982.

Arifin., Purwanto, B., Indarto, D., Wasita, B., Sumandjar, T., Poncorini, E. 2023. Degree of organ damage and inflammatory markers in sepsis mice models inducted by various doses of lipopolysaccharides [version 2; peer review: 2 not approved]. F1000Research. 12:5: 317 https://doi.org/10.12688/f1000research.128852.2.

Bermejo-Martin, J., Martín-Fernandez, M., López-Mestanza, C., Duque, P., Almansa, R., 2018. Shared features of endothelial dysfunction between sepsis and its preceding risk factors (aging and chronic disease). J. Clin. Med. 7 (11), 400 https://doi.org/10.3390/jcm7110400.

Bochon, B., Kozubska, M., Surygała, G., Witkowska, A., Kuźniewicz, R., Grzeszczak, W., Wystrychowski, G., 2019. Mesenchymal stem cells-potential applications in kidney diseases. Int. J. Mol. Sci. https://doi.org/10.3390/ijms20102462.

Bojalil, R., Ruíz-Hernández, A., Villanueva-Arias, A., Amezcua-Guerra, L.M., Casarez-Alvarado, S., Hernandez-Duenas, A.M., Rodriquez-Galicia, V., Pavon, L., Marquina, B., Becerril-Villanueva, E., Hernandez-Pando, R., Marquez-Velasco, R., 2023. Two murine models of sepsis: immunopathological differences between the sexes—possible role of TGFβ1 in female resistance to endotoxemia. Biol. Res. 56, 54. https://doi.org/10.1186/s40659-023-00469-8.

Dai, X., Zeng, Z., Fu, C., Zhang, S., Cai, Y., Chen, Z., 2015. Diagnostic value of neutrophil gelatinase-associated lipocalin, cystatin C, and soluble triggering receptor expressed on myeloid cells-1 in critically ill patients with sepsis-associated acute kidney injury. Crit. Care 19, 1–10. https://doi.org/10.1186/s13054-015-0941-6.

Fedchenko, N., Reifenrath, J., 2014. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. Diagn. Pathol. https://doi.org/10.1186/s13000-014-0221-9.

Gyawali, B., Ramakrishna, K., Dhamoon, A.S., 2019. Sepsis: the evolution in definition, pathophysiology, and management. SAGE Open Med. https://doi.org/10.1177/2050312119835043.

Harrell, C.R., Fellabaum, C., Jovicic, N., Djonov, V., Arsenijevic, N., Volarevic, V., 2019. Molecular mechanisms responsible for therapeutic potential of mesenchymal stem cell-derived secretome. Cells 8, 467. https://doi.org/10.3390/cells8050467.

Kiyonaga, N., Moriyama, T., Kanmura, Y., 2020. Effects of dexmedetomidine on lipopolysaccharide-induced acute kidney injury in rats and mitochondrial function in cell culture. Biomed. Pharmacother. 125 https://doi.org/10.1016/j.biopha.2020.109912.

Leng, Z., Zhu, R., Hou, W., Feng, Y., Yang, Y., Han, Q., Shan, G., Meng, F., Du, D., Wang, S., Fan, J., Wang, W., Deng, L., Shi, H., Li, H., Hu, Z., Zhang, F., Gao, J., Liu, H., Li, X., Zhao, Y., Yin, K., He, X., Gao, Z., Wang, Y., Yang, B., Jin, R., Stambler, I., Lim, L. W., Su, H., Moskalev, A., Cano, A., Chakrabarti, S., Min, K.J., Ellison-Hughes, G., Caruso, C., Jin, K., Zhao, R.C., 2020. Transplantation of ACE2-mesenchymal stem cells improves the outcome of patients with covid-19 pneumonia. Aging Dis. 11, 216–228. doi: 10.14336/AD.2020.0228.

Liu, X., Su, C., Xu, J., Zhou, D., Yan, H., Li, W., Chen, G., Zhang, N., Xu, D., Hu, H., 2019. Immunohistochemical analysis of matrix metalloproteinase-9 predicts papillary thyroid carcinoma prognosis. Oncol. Lett. 17 (2), 2308–2316. https://doi.org/10.3892/ol.2018.9850.

Madrigal, M., Rao, K.S., Riordan, N.H., 2014. A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. J. Transl. Med. 12, 1–14. https://doi.org/10.1186/s12967-014-0260-8.

Martínez, M.L., Ferrer, R., Torrents, E., Guillamat-Prats, R., Gomà, G., Suárez, D., Álvarez-Rocha, L., Pozo Laderas, J.C., Martín-Loeches, I., Levy, M.M., Artigas, A., 2017. Impact of source control in patients with severe sepsis and septic shock. Crit. Care Med. 45, 11–19. https://doi.org/10.1097/CCM.0000000000002011.

Mizuno, S., Nakamura, T., 2012. Improvement of sepsis by hepatocyte growth factor, an anti-inflammatory regulator: emerging insights and therapeutic potential. Gastroenterol. Res. Pract. 2012 https://doi.org/10.1155/2012/909350.

Niño, M.E., Serrano, S.E., Niño, D.C., Mccosham, D.M., Cardenas, M.E., Villareal, V.P., Lopez, M., Pazin-Filho, A., Jaimes, F.A., Cunha, F., Schulz, R., Torres-Dueñas, D., 2017. TIMP1 and MMP9 are predictors of mortality in septic patients in the emergency department and intensive care unit unlike MMP9/TIMP1 ratio: multivariate model. PLoS One 12. https://doi.org/10.1371/journal.pone.0171191.

Office of Laboratory Animal Welfare, 2002. Care and Use Institutional Animal Care and Use. section B 43–49.

Parajuli, B., Sonobe, Y., Kawanokuchi, J., Doi, Y., Noda, M., Takeuchi, H., Mizuno, T., Suzumura, A., 2012. GM-CSF increases LPS-induced production of proinflammatory mediators via upregulation of TLR4 and CD14 in murine microglia. J. Neuroinflammation 9, 1–12. https://doi.org/10.1186/1742-2094-9-268.

Pedrazza, L., Cubillos-rojas, M., de Mesquita, F.C., Luft, C., Cunha, A.A., Rosa, J.L., de Oliveira, J.R., 2017. Mesenchymal stem cells decrease lung inflammation during sepsis, acting through inhibition of the MAPK pathway. Stem Cell Res. Ther. 8, 1–14. https://doi.org/10.1186/s13287-017-0734-8.

Peerapornratana, S., Manrique-Caballero, C.L., Gómez, H., Kellum, J.A., 2019. Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. Kidney Int. 96, 1083–1099. https://doi.org/10.1016/j.kint.2019.05.026.

Renaldi, K., Simadibrata, M., Rahadiani, N., Handjari, D.R., Harahap, A.R., Harimurti, K., Zubir, N., Siregar, L., Loho, I.M., Suzanna, E., Prawirohardjo, B., Hidajat, H., Widodo, B., Rahniayu, A., Tambun, R., William, A., Makmun, D., 2023. The expressions of NF-κB, COX-2, Sp1, and c-Jun in pancreatic ductal adenocarcinoma and their associations with patient survival. Pathophysiology 30, 92–109. https://doi.org/10.3390/pathophysiology30020009.

Rhodes, A., Evans, L.E., Alhazzani, W., Levy, M.M., Antonelli, M., Ferrer, R., Kumar, A., Sevransky, J.E., Sprung, C.L., Nunnally, M.E., Rochwerg, B., Rubenfeld, G.D., Angus, D.C., Annane, D., Beale, R.J., Bellinghan, G.J., Bernard, G.R., Chiche, J.D., Coopersmith, C., de Backer, D.P., French, C.J., Fujishima, S., Gerlach, H., Hidalgo, J. L., Hollenberg, S.M., Jones, A.E., Karnad, D.R., Kleinpell, R.M., Koh, Y., Lisboa, T.C., MacHado, F.R., Marini, J.J., Marshall, J.C., Mazuski, J.E., McIntyre, L.A., McLean, A. S., Mehta, S., Moreno, R.P., Myburgh, J., Navalesi, P., Nishida, O., Osborn, T.M., Perner, A., Plunkett, C.M., Ranieri, M., Schorr, C.A., Seckel, M.A., Seymour, C.W., Shieh, L., Shukri, K.A., Simpson, S.Q., Singer, M., Thompson, B.T., Townsend, S.R., van der Poll, T., Vincent, J.L., Wiersinga, W.J., Zimmerman, J.L., Dellinger, R.P., 2017. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. Crit. Care Med. https://doi.org/10.1097/CCM.0000000000002255.

Salah, B., 2020. Staining haematoxylin & eosin. doi: 10.13140/RG.2.2.33261.72165.

Savoia, C., Sada, L., Zezza, L., Pucci, L., Lauri, F.M., Befani, A., Alonzo, A., Volpe, M., 2011a. Vascular inflammation and endothelial dysfunction in experimental hypertension. Int. J. Hypertens. https://doi.org/10.4061/2011/281240.

Savoia, C., Sada, L., Zezza, L., Pucci, L., Lauri, F.M., Befani, A., Alonzo, A., Volpe, M., 2011b. Vascular inflammation and endothelial dysfunction in experimental hypertension. Int. J. Hypertens. 2011 https://doi.org/10.4061/2011/281240.

Siddiqui, A.H., Valecha, G., Modi, J., Saqib, A., Weerasinghe, C., Siddiqui, F., el Sayegh, S., 2020. Predictors of 15-day survival for the intensive care unit patient on continuous renal replacement therapy: a retrospective analysis. Cureus. https://doi.org/10.7759/cureus.8175.

Steinhagen, F., Schmidt, S.V., Schewe, J.C., Peukert, K., Klinman, D.M., Bode, C., 2020. Immunotherapy in sepsis - brake or accelerate? Pharmacol. Ther. 208, 107476 https://doi.org/10.1016/j.pharmthera.2020.107476.

Su, V.Y.F., Lin, C.S., Hung, S.C., Yang, K.Y., 2019. Mesenchymal stem cell-conditioned medium induces neutrophil apoptosis associated with inhibition of the NF-κb pathway in endotoxin- induced acute lung injury. Int. J. Mol. Sci. 20 https://doi.org/10.3390/ijms20092208.

van der Poll, T., van de Veerdonk, F.L., Scicluna, B.P., Netea, M.G., 2017. The immunopathology of sepsis and potential therapeutic targets. Nat. Rev. Immunol. 17, 407–420. https://doi.org/10.1038/nri.2017.36.

Wajda, J., Dumnicka, P., Maraj, M., Ceranowicz, P., Kuźniewski, M., Kuśnierz-Cabala, B., 2019. Potential prognostic markers of acute kidney injury in the early phase of acute pancreatitis. Int. J. Mol. Sci. 20, 1–20. https://doi.org/10.3390/ijms20153714.

Wang, K., Xie, S., Xiao, K., Yan, P., He, W., Xie, L., 2018. Biomarkers of sepsis-induced acute kidney injury. Biomed Res. Int. 2018 https://doi.org/10.1155/2018/6937947.

Woźnica, E., Inglot, M., Woźnica, R., Łysenko, L., 2018. Liver dysfunction in sepsis. Adv. Clin. Exp. Med. 27, 547–552. https://doi.org/10.17219/acem/68363.

Yim, H.E., Kim, D.S., Chung, H.C., Shing, B., Moon, K.H., George, S.K., Kim, M.W., Atala, Z., Kim, J.H., Ko, I.K., Yoo, J.J., 2019. Controlled delivery of stem cell-derived trophic factors accelerates kidney repair after renal ischemia-reperfusion injury in rats. Stem Cells Transl. Med. 8, 959–970. https://doi.org/10.1002/sctm.18-0222.

Zakiyanov, O., Kalousová, M., Zima, T., Tesař, V., 2019. Matrix metalloproteinases in renal diseases: a critical appraisal. Kidney Blood Press. Res. 44, 298–330. https://doi.org/10.1159/000499876.