

# The miR-15a affected the development of sepsis and septic shock by suppression BCL-2

Yunzhen Wu (✉ [wuyunzhen285@163.com](mailto:wuyunzhen285@163.com))

Dongying People's Hospital <https://orcid.org/0000-0001-9797-2532>

Yuanli Xie

Dongying People's Hospital

Fangfang Jiao

Dongying People's Hospital

Xinlei Liu

Dongying People's Hospital

---

## Research article

**Keywords:** sepsis; septic shock; miR-15a; Bcl-2; cardiomyocyte; apoptosis

**Posted Date:** October 1st, 2019

**DOI:** <https://doi.org/10.21203/rs.2.15376/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

**Background:** This study aimed to investigate the mechanism of microRNA-15a (miR-15a) in the development of sepsis and septic shock.

**Methods:** Sepsis and septic shock rat models were constructed by intraperitoneal injection of *E. coli* endotoxin (LPS). The real-time polymerase chain reaction (RT-PCR), Enzyme-Linked ImmunoSorbent Assay (ELISA), ematoxylin-eosin (HE) and Masson staining, TdT-mediated dUTP Nick-End Labeling (TUNEL), as well as Western blot analysis were performed to reveal the expression of microRNA-15a and changes in sepsis/septic shock myocardial cells or tissue. The rat sepsis model (sepsis group and septic shock group) was successfully established.

**Results:** The results of HE and Mason staining showed that myocardial tissue damage gradually deepened with the progression of sepsis. Moreover, the serum levels of creatinine kinase-mb (CK-MB) and cardiac troponin I (cTnI) in model groups were significantly increased than those in control group. In addition, the RT-PCR analysis showed that miR-15a was up-regulated in model groups. Furthermore, luciferase reporter gene assay showed that 3'-UTR was the binding site of BCL-2 to miR-15a. Finally, the TUNEL and Western blot showed the cardiomyocyte apoptosis in model group.

**Conclusions:** The overexpression of miR-15a might take part in the progression of LPS-induced sepsis and septic shock via suppressing Bcl-2 expression. Furthermore, myocardial markers such as CK-MB and cTnI might be biomarkers for sepsis progression.

## Background

Sepsis is life threatening organ dysfunction caused by a dysregulated host response to infection <sup>1</sup>. As the subset of sepsis, septic shock is defined in which underlying circulatory and cellular or metabolic abnormalities, and are profound enough to increase mortality substantially <sup>2</sup>. Generally, 40–60% sepsis patients have multiple organ dysfunction <sup>3</sup>. Among these, myocardial dysfunction is one of the main predictors of poor outcome in septic patients, with mortality rates next to 70% <sup>4</sup>. Although novel therapeutic pathways targeting organ dysfunction hold renewed promise for septic, the latest estimates of incidence in the United States, Europe, and the United Kingdom is as high as 1/1000 of the population <sup>5</sup>. Therefore, studying the pathogenesis of sepsis and its subset septic shock is vital for the clinical treatment.

Myocardial inhibition is a recognized manifestation of organ dysfunction in sepsis, and myocardial apoptosis is a key component of its development <sup>6</sup>. During this process, RNA (microRNAs) are commonly used as fingerprints for sepsis and proved to be potential septic biomarkers <sup>7</sup>. The overexpression of miRNAs increase lipopolysaccharide (LPS)-induced rat survival and inhibit LPS-induced cardiomyocyte apoptosis <sup>8</sup>, which can be used as an independent therapeutic target for sepsis <sup>9</sup>. As a member of miRNAs, *miR-15a* is confirmed to participate in a variety of biological processes including proliferation

and apoptosis<sup>10,11</sup>. Wang et al. indicated that the up-regulation of miR-15a inhibited LPS-induced biological pathways in sepsis<sup>12</sup>. Not only *miR-15a* can be used as a biomarker to distinguish sepsis from systemic inflammatory response syndrome (SIRS)<sup>13</sup>, but bacterial infection and/or bacterial LPS increase the level of *miR-15a/16* in macrophages<sup>14</sup>. In clinical treatment, myocardial depression is always combined with the variation of indicators such as cardiac troponin I (cTnI), procalcitonin (PCT) and creatinine kinase-mb (CK-MB) in patients until death or recovery<sup>15,16</sup>. Importantly, a previous study shows that myocardial apoptosis-regulating gene B cell lymphoma 2 (Bcl-2) might be used as an indicator during the process of myocardial injury in sepsis<sup>17</sup>. Members of Bcl-2 gene family have a central role in regulating programmed cell death by controlling pro-apoptotic and anti-apoptotic intracellular signals<sup>18</sup>. In previous study, the mice model has been successfully used to examine the role of Bcl-2 family members on the regulation of sepsis induced splenic dendritic cells and macrophages survival<sup>19</sup>. Thus, selective inhibition of specific anti-apoptotic Bcl-2 family proteins represents an exciting therapeutic opportunity for sepsis and septic shock. However, the biological function of Bcl-2 and *miR-15a* in sepsis and septic shock had not yet been thoroughly studied.

In this study, the LPS-induced sepsis and septic shock rat model were established. The real-time polymerase chain reaction (RT-PCR), Enzyme-Linked ImmunoSorbent Assay (ELISA), ematoxylin-eosin (HE) and Masson staining, TdT-mediated dUTP Nick-End Labeling (TUNEL), as well as Western blot analysis were performed to reveal the expression of microRNA-15a and changes in myocardial cells or tissue. Our findings may reveal the detail roles of miR-15a on neural damages of sepsis and septic shock, and provide new insights into the underlying mechanisms responsible for the treatment of sepsis and septic shock.

## Methods

### Rat model construction and grouping

A total of 60 male Sprague Dawley (SD) rats (180–200 g, 6 weeks, SPF grade) were purchased from Experimental Animal Center of the Chinese Academy of Sciences. After one week standard feeding, all rats were randomly divided into control group (n = 12, intraperitoneal injection of 10 mg/kg PBS), sepsis group (n = 24, intraperitoneal injection of 10 mg/kg LPS (O55:B5; Sigma, St Louis, MO, USA)) and septic shock group (n = 24, intraperitoneal injection of 10 mg/kg LPS (O55:B5; Sigma, St Louis, MO, USA)). Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) (monitored by a pressure transducer) were used to calculate mean arterial pressure (MAP) with  $MAP = DBP + (1/3 (SBP - DBP))$ . Compared with the baseline value, the MAP value decreased by less than 30%, equal to 30% and more than 30% was considered as sepsis model, shock model and septic shock model respectively. This study was approved by the ethics committee of Dongying People's Hospital (Approval number: dy2018025), and all experiments were in accordance with the guide for the care and use of laboratory animals established by United States National Institutes of Health (Bethesda,

MD, USA). All the animal experiments were conducted in the Science and Education Building of Dongying People's Hospital.

## Specimen collection

Blood (4ml/each sample) was collected from the common carotid artery intubation. One sample of blood was treated with anticoagulant (heparin) and centrifuged to obtain plasma. The other sample of natural liquid was precipitated by centrifugation to obtain serum. The plasma and serum were separately obtained for the detecting biochemical indicators. After the rats were killed, the heart tissues were removed and fixed at 10% formaldehyde with the size of 1.0 mm<sup>3</sup>. The rest of the heart tissues were stored at -80 °C.

## CK-MB, cTnI and PCT detection

The CK-MB and PCT of the three groups were measured by ELISA based on the kit instructions (Wuhan Yousheng Technology Co., Ltd., R&D Company of the United States). Meanwhile, the cTnI levels of three groups were measured using a Beckman-Coulter ACCESS 2 automatic microparticle chemiluminescence immunoassay analyzer. All specific operations were strictly in accordance with the instructions.

## RT-PCR assay

Total RNAs were extracted from cells in different groups using TRizol reagent (Invitrogen, USA), and reverse transcribed using MBI (San Francisco, CA, USA). Ultraviolet spectrophotometer was used to determine the concentration and purity of the RNA. The qRT-PCR was performed on ABI7500 (Thermo Fisher Scientific), and the amplification primer sequences of each gene and their primers were shown in Table 1. In addition, the reaction conditions of RT-PCR experiments were as follows: 95°C for 10s, 60°C for 20 s, 72°C for 34 s, 40 cycles. The relative expression of miRNA was calculated using the  $2^{-\Delta\Delta Ct}$  method <sup>20</sup>.

## Western blot assay

The expression of proteins in myocardial cell were detected by Western blot. Total proteins of myocardial cell from different groups were extracted by RIPA lysis buffer (Thermo Scientific, USA), and determined by Bradford method. After centrifugation, proteins were separated by 10% SDS-polyacrylamide gel electrophoresis, and transferred to a polyvinylidene fluoride membrane (Roche). Then the membrane was blocked with 50g/L skim milk for 2 h, and incubated with goat anti-rat primary antibody (1:5000, Abcam, USA) overnight at 4°C. Then, the membrane was incubated with HRP-labeled secondary antibody IgG

(1:5000, Jinqiao Biotechnology, China) for 2 h at 37°C. Finally, the protein was colored by DAB and imaged by image analysis system for analysis.

After the study, all rats were euthanized. The right hand held the rat tail and pull it back, and the left thumb and forefinger pressed down firmly on the rat head at the same time. The external force was used to dislocate the cervical spine of the rat, and the spine and the brain were disconnected. This method can quickly lose consciousness and reduce pain of experimental animals, which is a commonly used method for euthanasia of small experimental animals.

## HE staining

After the rats were executed, the right hind limb was fixed with 10% neutral formaldehyde solution for 24h. Then the decalcification process was performed for 30 days by using 10% Ethylenediaminetetraacetic acid (EDTA), followed by routine dehydration, transparency, paraffin immersion, embedding and coronal section. The sections were dewaxed and dehydrated with ethanol of different gradient concentration, then stained with Hematoxylin-eosin (HE). The pathological changes were observed under light microscope.

## Masson staining

The paraffin sections need to undergo xylene dewaxing and alcohol gradient dehydration before they were subjected to Masson staining. Then, the sections were dyed with saturated alcohol bitter acid for 1h, and hematoxylin stained for 5–10min. After differentiation by hydrochloric acid and alcohol, the sections were stained with Ponceau acid fuchsin solution for 10 minutes and 1% phosphomolybdic acid for 5 minutes in turn, and then they were immersed in bright green solution. After gradient alcohol dehydration and xylene infiltration, the paraffin sections were sealed by neutral resin. The change of collagen content was observed under light microscope, the collagen and non-collagen components were distinguished by gradation adjustment. Moreover, the HP IAS2000 computer image analysis system was used to measure myocardial collagen volume fraction (CVF) and myocardial perivascular collagen area (PCVA).

## Detection of cell apoptosis

TUNEL assay was performed to detect the myocardial cell apoptosis in rats from each group. The rat heart was fixed with 10% formaldehyde, embedded in paraffin, then dewaxed and hydrated with a gradient of ethanol. DNA 3-OH terminal was labeled by terminal deoxynucleotidyl TUNEL, and apoptotic cells were detected by in situ fluorescence labeling. According to the TUNEL kit (ROCHE, Swiss) instructions, the apoptotic cardiomyocytes was with red fluorescence, while all nucleated cells was with blue fluorescence after DAPI staining. Finally, four fields of ischemic area were randomly selected, and

the total number of cells and the number of apoptotic cells in each field were counted, and the apoptotic index (AI) was calculated.

## Luciferase reporter gene assay

The target sites for Bcl-2 and *miR-15a* were determined by Target Scan, and the mutant and wild sequences were designed according to the predicted results. The Bcl-2 mutant sequence and the wild sequence fragment were cloned and bound to the PGL-3 vector. The mutated sequence was co-transfected into 293T cells with *miR-15a* mimics or the *miR-15a* negative control, and named as MT + mimics group and MT + NC group, respectively. In addition, 293T cells were transfected with wild-type sequences combined with *miR-15a* or its' negative control. After transfection for 48 hours, fluorescent activity of the cells was detected by luciferase kit (Yuanping Hao Biotechnology Co., Ltd., China).

## Statistical methods

Data analysis was performed using GraphPad Prism v5.01 statistical software, and all data were expressed as mean  $\pm$  standard deviation (SD). The comparison between groups was analyzed by t-test.  $P < 0.05$  was considered statistically significant.

## Result

### Hemodynamics in rats

The sepsis model and the shock model were successfully established at 12 and 24 hours LPS treatment, followed by the hemodynamics analysis. The result showed that the SBP, DBP, and MAP of rats in control group was  $127 \pm 4$  mmHg,  $75 \pm 3$  mmHg, and  $93 \pm 3$  mmHg, respectively. After LPS injection for 20 hours, the blood pressure was dropped by about 30%, and the rat entered the shock state. The SBP (Figure 1A), DBP (Figure 1B) and MAP (Figure 1C) levels after LPS injection were significantly lower than those of control group ( $P < 0.05$ ). Meanwhile, HR level (Figure 1D) was significantly higher than that of control group ( $P < 0.05$ ).

### Pathological changes of cardiac tissue

The results of HE staining showed that the myocardial structure of the control group was intact and clear. Meanwhile, the myocardial fibers were closely arranged and no obvious necrosis was observed in control group. The normal structure of the heart was destroyed, and the myocardial fibers were sparse and loosely arranged in sepsis group and septic shock group. Furthermore, the nucleus of myocardial cell was condensed and ruptured. Compared with the septic shock group, the degree of lesions was significantly reduced in sepsis group (Figure 2A).

Masson staining showed that the cardiomyocytes were arranged tightly and neatly, and the myocardial fibers were arranged tightly in the control group (Figure 2B). The degree of lesion in the sepsis group was significantly lower than that in the septic shock group ( $P < 0.05$ ). Compared with the control group, the CVF and PCVA level in the sepsis group and the septic shock group were increased significantly (all  $P < 0.05$ ). Furthermore, the level of CVF (Figure 2C) and PCVA (Figure 2D) in septic shock group was significantly higher than the sepsis group (all  $P < 0.05$ ).

## The expression of CK-MB, cTnI and PCT

Compared with control group, serum CK-MB (Figure 3A) and cTnI (Figure 3B) in sepsis group and septic shock group were significantly higher than those in control group (all  $P < 0.05$ ), and septic shock group was significantly higher than that in sepsis group ( $P < 0.05$ ). Furthermore, ELISA was used to detect changes in serum PCT content (Figure 3C). The results showed that the serum PCT level of the control group was very low, and the serum PCT level of the sepsis group was significantly higher than that of the control group ( $P < 0.05$ ). Meanwhile, the serum PCT level in septic shock group was significantly higher than that in sepsis group ( $P < 0.05$ ). These results suggested that microRNA-15a might involve in the pathogenic process from sepsis to septic shock, and the overexpression of microRNA-15a might decrease with the development of sepsis in rats.

## Expression of miR-15a and the association with Bcl-2

Real-time PCR was used to detect the expression of miR-15a in control group, sepsis group and septic shock group (Figure 4A). The result showed that compared with control group, the expression of miR-15a were significantly increased in both sepsis and septic shock group (all  $P < 0.05$ ). Meanwhile, compared with sepsis group, the expression of miR-15a was significantly lower in septic shock group ( $P < 0.05$ ).

The Target Scan results showed that the binding site of Bcl-2 and *miR-15a* was the 3'-UTR region. Additionally, there was no obvious difference in luciferase activity between MT + mimics group and MT + NC group, but the activity of luciferase in WT + mimics group was significantly lower than that in WT + NC group ( $P < 0.01$ , Figure 4B). These results indicated that miR-15a might affect myocardial apoptosis in sepsis and septic shock animals by targeting BCL-2.

## Changes of myocardial apoptosis index

The results of TUNEL assay showed that the apoptosis index of model group (sepsis group and septic shock group) was significantly higher than those of the control group (all  $P < 0.05$ ), while the apoptosis

index of septic shock was significantly higher than that of the sepsis group ( $P < 0.05$ ) (Figure 5A). Western blot results showed that the expression of glucose regulatory protein 78 (GRP78) in cardiac tissue of model group (sepsis group and septic shock group) was significantly higher than that of control group, while the expression of GRP78 in septic shock group was significantly higher than that in sepsis group (all  $P < 0.05$ ) (Figure 5B). Furthermore, after LPS injection, the expression of BCL-2 decreased significantly either between model group (sepsis group and septic shock group) and control group (all  $P < 0.05$ ), or between sepsis group and septic shock group ( $P < 0.05$ ) (Figure 5C).

## Discussion

Sepsis remains a common, expensive, and deadly problem throughout the world<sup>21</sup>. To explore the influencing factors such as miR-15a in the progression of sepsis, the rat sepsis model (sepsis group and septic shock group) was established in current study. The results of HE and Mason staining showed that myocardial tissue damage gradually deepened with the progression of sepsis. Moreover, the serum levels of CK-MB, cTnl and PCT in model groups were significantly increased than those in control group. In addition, the RT-PCR analysis showed that miR-15a was up-regulated in model groups. Furthermore, luciferase reporter gene assay showed that 3'-UTR was the binding site of BCL-2 to miR-15a. Finally, the TUNEL and Western blot showed the cardiomyocyte apoptosis in model group.

MiR-15a is a tumor suppressor that inhibiting cell proliferation and promote cell apoptosis in cancer progression. Serum miR-15a level can be used as a biomarker that distinguish sepsis from systemic inflammatory response syndrome in human subjects<sup>22</sup>. A previous study shows that miR-15a is up-regulated in the serum of neonatal sepsis patients and inhibits the LPS-induced inflammatory pathway<sup>23</sup>. Actually, the biological function of miR-15a is realized by targeting a variety of oncogenes including Bcl-2<sup>24</sup>. In chronic lymphocytic leukemia, miR-15a has a tumor suppressor function by targeting the Bcl-2 oncogene<sup>25</sup>. In fact, overexpression of Bcl-2 has a protective effect on apoptosis in septic cells<sup>26</sup>. A previous study indicates that the ability of Bcl-2 overexpression to improve survival in patients with sepsis is partly related to the prevention of changes in spleen transcriptional responses caused by sepsis<sup>27</sup>. Hu et al. showed that that the decreasing expression of Bcl-2 was beneficial to reduce cardiomyocyte apoptosis<sup>28</sup>, which further indicating that miR-15a affected cardiomyocyte apoptosis by regulating Bcl-2. In this study, the RT-PCR analysis showed that miR-15a was up-regulated in both sepsis group and septic shock group. Meanwhile, the luciferase reporter gene assay showed that the down-regulation of Bcl-2 was a target gene of the up-regulated miR-15a. Thus, we speculated that the overexpression of miR-15a might take part in the progression of sepsis and septic shock via suppressing Bcl-2 expression.

Apoptosis was a process of inductive destruction of non-necrotizing cells and suicide<sup>29</sup>. It play an important role in the pathogenesis of sepsis<sup>30</sup>. During this process, many proteins had been studied to have an apoptotic effect. GRP78 is a heat shock protein present in all cells and a widely used marker of endoplasmic reticulum stress<sup>31</sup>. Wei et al. suggested that blocking inflammatory responses and

inhibiting cardiomyocyte apoptosis by certain proteins including CTRP3 could affect the process of myocardial protein in septic rats<sup>32</sup>. Under hypoxic conditions, the presence of peptide-bound GRP78 can inhibit cardiomyocyte apoptosis<sup>33</sup>. In this study, the cardiomyocyte apoptosis index GRP78 was significantly up-regulated in sepsis and septic shock group, indicating that there might be apoptosis of cardiomyocytes in patients with sepsis and septic shock. Furthermore, a previous study indicates that the cute physiological can be performed on cTnI and CK-MB to evaluate the predictive value of cardiac markers in sepsis patients<sup>34</sup>. A Jain et al. revealed that cTnI was independent of the degree of septic myocardial dysfunction in patients with septic shock<sup>35</sup>. Combined detection of cTnI and CK-MB is proved to be more accurately in evaluating the progression of sepsis<sup>36</sup>. Wu et al. found that plasma levels of cTn1, CK-MB and PCT in the myocardial injury group were remarkably higher in children with sepsis myocardial injury than that in the non-myocardial injury group<sup>37</sup>. Since the CK-MB and cTnI levels were increased with the development of sepsis<sup>38,39</sup>, reducing serum CK-MB and cTnI levels was beneficial to improve myocardial damage in sepsis<sup>40</sup>. In the current study, the ELISA assay showed that the CK-MB and cTnI levels were highly expressed in sepsis and septic shock group. Thus, we speculated that myocardial markers including CK-MB and cTnI might be used as biomarkers for sepsis progression.

## Conclusions

In conclusion, the overexpression of miR-15a might take part in the progression of LPS-induced sepsis and septic shock via suppressing Bcl-2 expression. Furthermore, myocardial markers such as CK-MB and cTnI might be biomarkers for sepsis progression.

## Abbreviations

The real-time polymerase chain reaction RT-PCR

Enzyme-Linked ImmunoSorbent Assay ELISA

ematoxylin-eosin HE

TdT-mediated dUTP Nick-End Labeling TUNEL

## Declarations

Ethics approval and consent to participate: This study was conducted after obtaining approval of Dongying People's Hospital's ethical committee (Approval number: dy2018025).

Consent for publication: Not applicable.

Availability of data and material: All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests: The authors declare that they have no competing interests.

Funding: Not applicable.

Authors' contributions: YZW and YLX designed and analyzed the experiment, and was a major contributor in writing the manuscript. FFJ and XLL performed the experiment. All authors read and approved the final manuscript.

Acknowledgements: Not applicable.

## References

1. Delano MJ, Ward PA. Sepsis-induced immune dysfunction: can immune therapies reduce mortality? *Journal of Clinical Investigation*. 2016;126:23.
2. Abraham E. New Definitions for Sepsis and Septic Shock: Continuing Evolution but With Much Still to Be Done. *Jama*. 2016;315:757.
3. Kotecha A, Vallabhajosyula S, Coville HH, Kashani K. Cardiorenal syndrome in sepsis: A narrative review. *Journal of Critical Care*. 2018;43:122–7.
4. Romerobermejo FJ, Ruizbailen M, Gilcebrian J, Huertosranchal MJ. Sepsis-induced Cardiomyopathy. *Current Cardiology Reviews*. 2011;7:163–83.
5. Gotts JE, Matthay MA. Sepsis: pathophysiology and clinical management. *BMJ*. 2016;353.
6. Bo Z, Yue L, Jin-Sheng Z, Xiao-Hui Z, Wen-Jia C, Xin-Hua Y, et al. Cortistatin protects myocardium from endoplasmic reticulum stress induced apoptosis during sepsis. *Molecular & Cellular Endocrinology*. 2015;406:40–8.
7. Goodwin AJ, Guo C, Cook JA, Wolf BJ, Halushka PV, Fan H. Plasma levels of microRNA are altered with the development of shock in human sepsis: an observational study. *Critical Care*. 2015;19:440-.
8. Yao Y, Sun F, Lei M. miR–25 inhibits sepsis-induced cardiomyocyte apoptosis by targetting PTEN. *Bioscience Reports*. 2018;38.
9. Ling L, Zhang SH, Zhi LD, Li H, Wen QK, Li G, et al. MicroRNA–30e promotes hepatocyte proliferation and inhibits apoptosis in cecal ligation and puncture-induced sepsis through the JAK/STAT signaling pathway by binding to FOSL2. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*. 2018;104:411.
10. Liu N, Jiao T, Huang Y, Liu W, Li Z, Ye X. Hepatitis B Virus Regulates Apoptosis and Tumorigenesis through the MicroRNA–15a-Smad7-Transforming Growth Factor Beta Pathway. *Journal of Virology*. 2015;89:2739–49.

11. Huijuan W, Pengjun Z, Weijun C, Dan F, Yanhong J, Lixin X. Serum microRNA signatures identified by Solexa sequencing predict sepsis patients' mortality: a prospective observational study. *Plos One*. 2012;7:e38885.
12. Wang X, Liu X, Xu J, Hou S, Zhang X, Ding Y. miR-15a/16 are upregulated in the serum of neonatal sepsis patients and inhibit the LPS-induced inflammatory pathway. *International Journal of Clinical & Experimental Medicine*. 2015;8:5683-90.
13. Huijuan W, Pengjun Z, Weijun C, Dan F, Yanhong J, Li-Xin X. Evidence for serum miR-15a and miR-16 levels as biomarkers that distinguish sepsis from systemic inflammatory response syndrome in human subjects. *Clinical Chemistry & Laboratory Medicine*. 2012;50:1423-8.
14. Hyung-Geun M, Jincheng Y, Yijie Z, Yang J. miR-15a/16 regulates macrophage phagocytosis after bacterial infection. *Journal of Immunology*. 2014;193:4558.
15. Court O, Kumar A, Parrillo JE, Kumar A. Clinical review: Myocardial depression in sepsis and septic shock. *Critical Care*. 2002;6:500-8.
16. Apple FS, Murakami MM. Cardiac Troponin and Creatine Kinase MB Monitoring during In-Hospital Myocardial Reinfarction. *Clinical Chemistry*. 2005;51:460-3.
17. Zhai CS, Tang H, Liang YB, Chen ZB, Li ZY, Ma ZF. [Study of the effect of p53 and Bcl-2 protein expression in myocardial apoptosis in septic rats]. 2008;20:733.
18. Ashkenazi A, Fairbrother WJ, Levenson JD, Souers AJ. From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. *Nature Reviews Drug Discovery*. 2017;16:273-84.
19. Peckpalmer OM, Unsinger J, Chang K, McDonough JS, Perlman H, McDunn JE, et al. Modulation of the Bcl-2 family blocks sepsis-induced depletion of dendritic cells and macrophages. *Shock*. 2009;31:359-66.
20. Livak KJ ST. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2(-Delta Delta C(T)) Method. *METHODS*. 2001;Dec;25(4):402-8.
21. Kwon OK, Lee W, Kim SJ, Lee YM, Lee JY, Kim JY, et al. In-depth proteomics approach of secretome to identify novel biomarker for sepsis in LPS-stimulated endothelial cells. *Electrophoresis*. 2016;36:2851-8.
22. Wang H, Zhang P, Chen W, Feng D, Jia Y, Xie L. Evidence for serum miR-15a and miR-16 levels as biomarkers that distinguish sepsis from systemic inflammatory response syndrome in human subjects. *Clinical Chemistry and Laboratory Medicine*. 2012;50:1423-8.
23. Wang X, Wang X, Liu X, Wang X, Xu J, Hou S, et al. miR-15a/16 are upregulated in the serum of neonatal sepsis patients and inhibit the LPS-induced inflammatory pathway. *International Journal of Clinical & Experimental Medicine*. 2015;8:5683-90.

24. R I A, G A C, C M C. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. *Cell Death and Differentiation*. 2010;17:215.
25. Calin GA, Amelia C, Muller F, Manuela F, Wojcik SE, Masayoshi S, et al. MiR-15a and miR-16-1 cluster functions in human leukemia. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;105:5166-71.
26. Hotchkiss RS, Swanson PE, Knudson CM, Chang KC, Cobb JP, Osborne DF, et al. Overexpression of Bcl-2 in transgenic mice decreases apoptosis and improves survival in sepsis. *Journal of Immunology*. 1999;162:4148-56.
27. Wagner TH, Drewry AM, Macmillan S, Dunne WM, Chang KC, Karl IE, et al. Surviving sepsis: bcl-2 overexpression modulates splenocyte transcriptional responses in vivo. *American Journal of Physiology Regulatory Integrative & Comparative Physiology*. 2007;292:R1751.
28. Dandan HU, Lou L, Huilian XU. Effect of Shenfu Injection on the Cardiomyocyte Apoptosis and the Expression of Bcl-2 in Rat with Sepsis. *Journal of Emergency in Traditional Chinese Medicine*. 2015.
29. Nevière R, Marchetti P. Does Apoptosis Play a Role in the Development of Sepsis-Induced Myocardial Dysfunction? *Update in Intensive Care & Emergency Medicine*. 2002;38:124-36.
30. Hai-Yan Y, Jian-Rui W, Rui Z, Xiao-Ling Y, You-Feng Z, Wei-Jian L. Effect of glutamine on caspase-3 mRNA and protein expression in the myocardium of rats with sepsis. *American Journal of the Medical Sciences*. 2014;348:315.
31. Gonzalez GM, Selim MJ, Pizzo SV. GRP78: a multifunctional receptor on the cell surface. *Antioxidants & Redox Signaling*. 2009;11:2299-306.
32. Wei WY, Ma ZG, Zhang N, Xu SC, Yuan YP, Zeng XF, et al. Overexpression of CTRP3 protects against sepsis-induced myocardial dysfunction in mice. *Molecular & Cellular Endocrinology*. 2018;476.
33. Hardy B, Raiter A. Peptide-binding heat shock protein GRP78 protects cardiomyocytes from hypoxia-induced apoptosis. *Journal of Molecular Medicine*. 2010;88:1157-67.
34. Zang X, Chen W, Sheng B, Zhao L, Gu X, Zhen J, et al. [Predictive value of early phrase echocardiography and cardiac biological markers in patients with severe sepsis: a five-year single-center retrospective study]. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue*. 2018;30:332-6.
35. Jain A, Sankar J, Anubhuti A, Yadav DK, Sankar MJ. Prevalence and Outcome of Sepsis-induced Myocardial Dysfunction in Children with 'Sepsis' 'With' and 'Without Shock'-A Prospective Observational Study. *J Trop Pediatr*. 2018.
36. Hua H, Wang GD, Equipment DO, Hospital LT. Clinical value of combined detection of cTnI and CKMB in sepsis patients. *Hainan Medical Journal*. 2016.

37.Wu ZJ, Chen YF, Wang HD, Gao FH. [Expression of plasma miRNA-497 in children with sepsis-induced myocardial injury and its clinical significance]. Zhongguo dang dai er ke za zhi = Chinese journal of contemporary pediatrics. 2018;20:32.

38.Luo Y, Tan Z, Xinjun LI, Weili XU. Effect discussion of serum cTnl, CK-MB, NT-proBNP joint test in children with sepsis. China Modern Doctor. 2016.

39.Huang J, Liu S, Qunyan LE, Emergency DO. Clinical significance of serum procalcitonin and cardiac troponin I in patients with sepsis. China Modern Doctor. 2017.

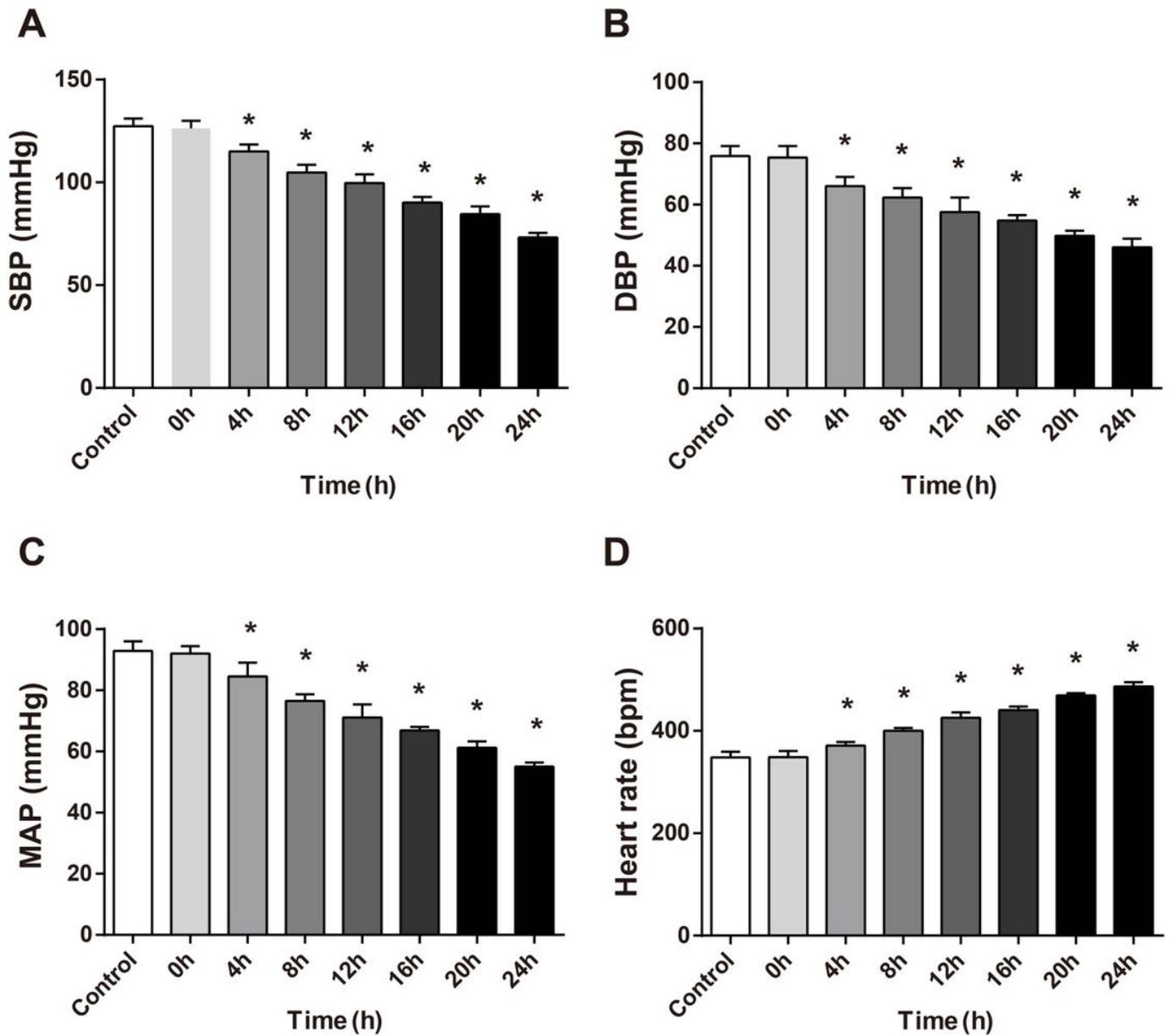
40.Song Q, Fan R, Zhu H. Study on Myocardial Injury and Recovery of Clear Toxin Extract Liquid from Rats with Sepsis. Journal of Emergency in Traditional Chinese Medicine. 2018.

## Table 1

**Table 1.** PCR primer sequences (5'-3')

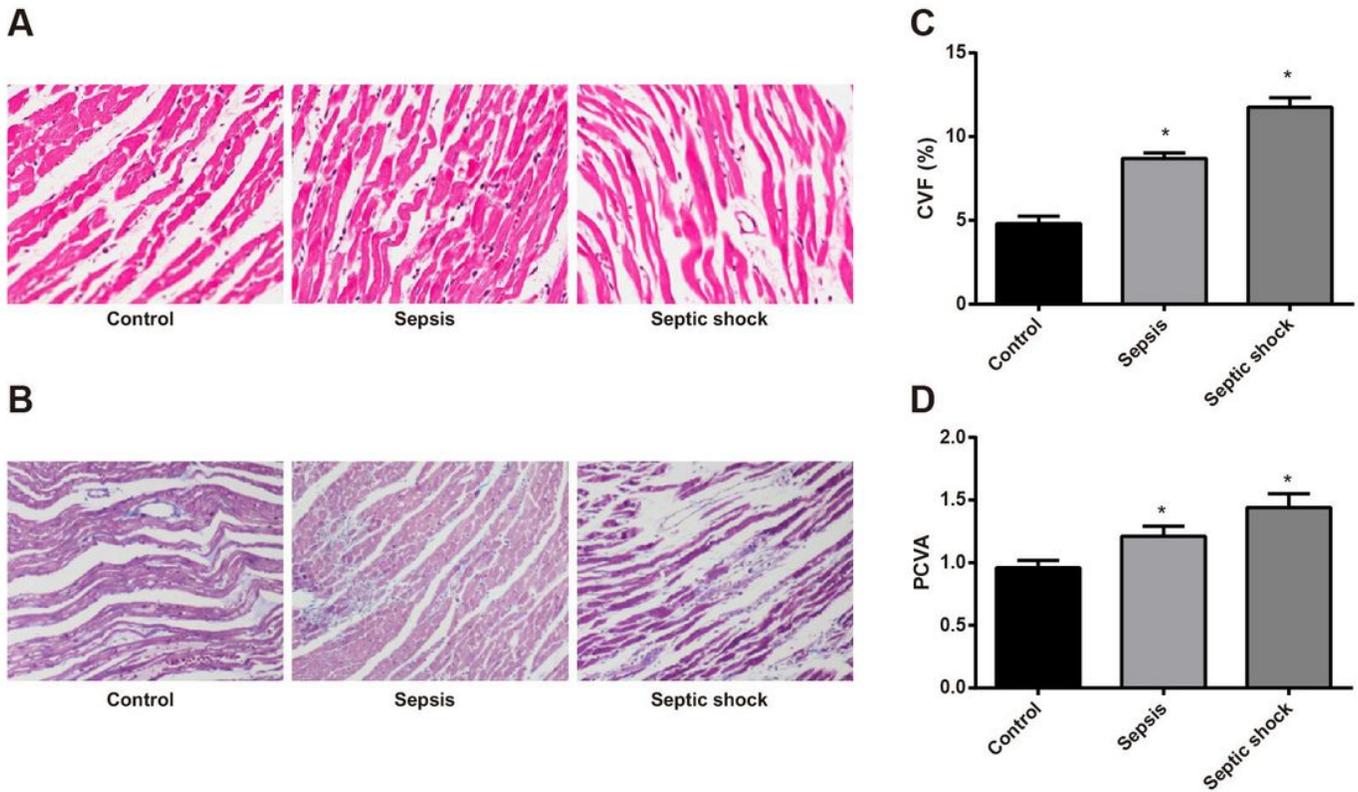
Name of primer	Sequences
<i>miR-15a</i> F	GATACTCGAGCAGAAGTTTGGCTAATTTAATAA
<i>miR-15a</i> R	GCGAATTCGCCAAGGATGACCTTAAGCCTC
U6 F	CTCGCTTCGGCAGCACA
U6 R	AACGCTTCACGAATTTGCGT

## Figures



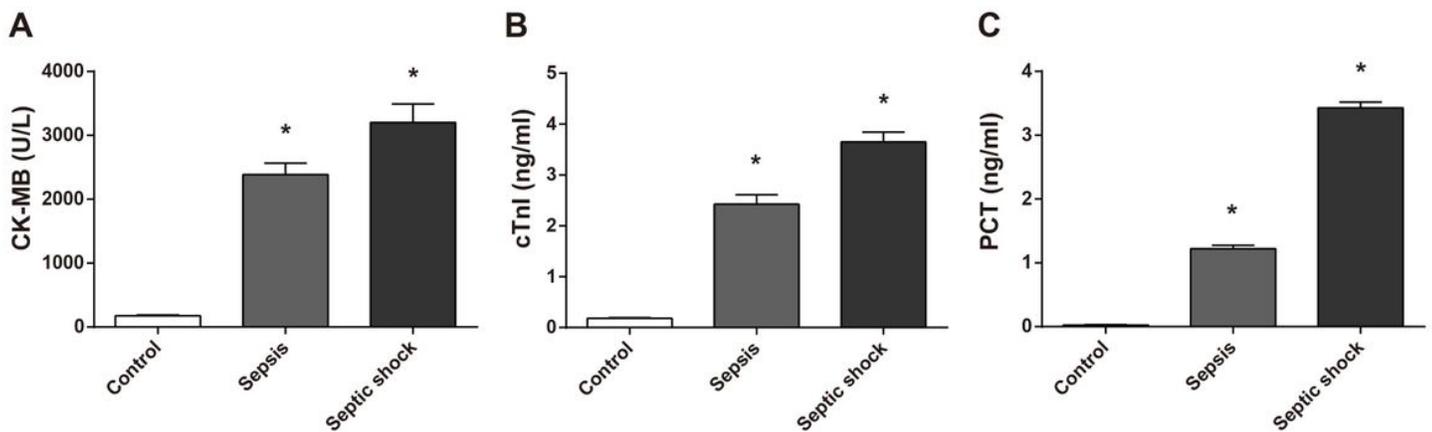
**Figure 1**

Changes of blood pressure and heart rate in rats after lipopolysaccharide (LPS) injection. A, changes in systolic blood pressure; B, changes in diastolic blood pressure; C, changes in mean arterial pressure (MAP); D, changes in heart rate. \*,  $P < 0.05$ , compared with control group.



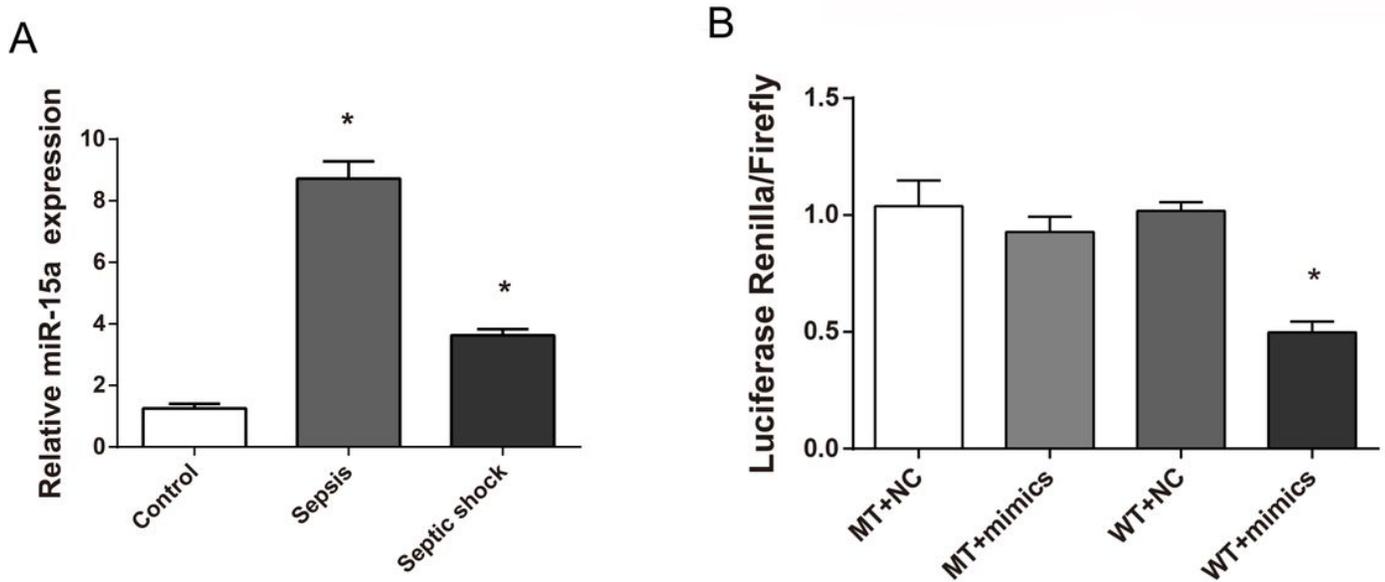
**Figure 2**

The results of myocardial pathological changes and myocardial collagen analysis. A, Ematoxylin-eosin (HE) staining; B, Masson staining; C, quantitative analysis of myocardial collagen volume fraction; D, quantitative analysis of intravascular peri vascular collagen area. \*,  $P < 0.05$ , compared with control group.



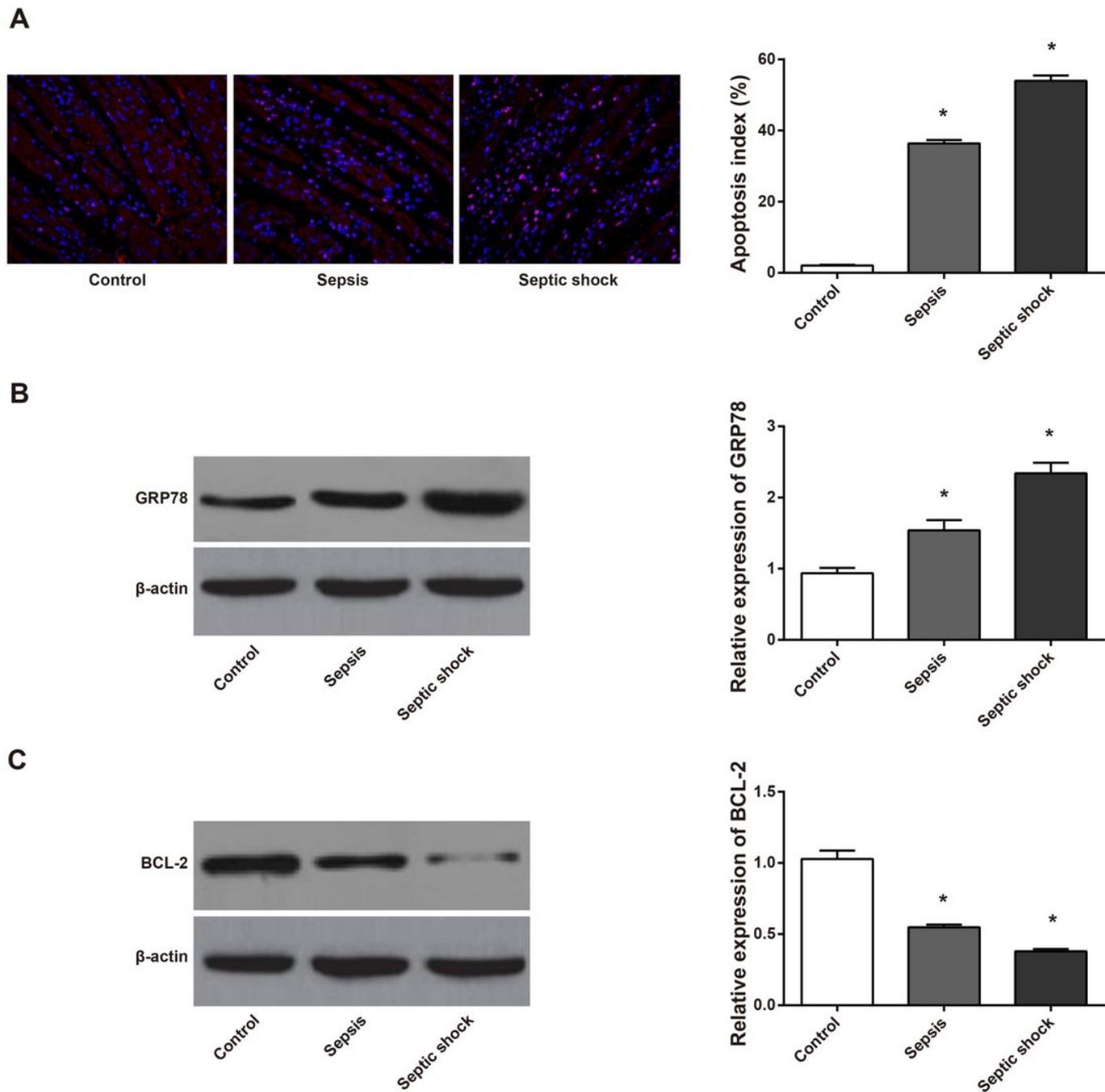
**Figure 3**

Changes of myocardial markers in rats. A, creatinine kinase-mb (CK-MB); B, cardiac troponin I (cTnl); C, procalcitonin (PCT). \*,  $P < 0.05$ , compared with control group.



**Figure 4**

Expression of microRNA-15a and the association with B cell lymphoma 2 (Bcl-2) in myocardial tissue. A, changes of microRNA-15a level in myocardium of rats in each group; \*,  $P < 0.05$ , compared with control group. B, luciferase reporter gene activity assay; \*,  $P < 0.05$ , compared with WT + NC group.



**Figure 5**

changes of myocardial cell apoptosis indexes in rats. A, apoptosis results; B, glucose regulatory protein (GRP) 78 expression level; C, Bcl-2 expression level. \*,  $P < 0.05$ , compared with control group.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [NC3RsARRIVEGuidelinesChecklistfillable.pdf](#)