

SUPPLEMENTAL DATA

Ribonuclease 1 attenuates septic cardiomyopathy and cardiac apoptosis in a murine model of polymicrobial sepsis

Elisabeth Zechendorf¹, Caroline E. O’Riordan², Lara Stiehler¹, Natalie Wischmeyer¹, Fausto Chiazza³, Debora Collotta³, Bernd Denecke⁴, Sabrina Ernst⁵, Gerhard Müller-Newen⁵, Sina M. Coldewey^{6,7}, Bianka Wissuwa^{6,7}, Massimo Collino³, Tim-Philipp Simon¹, Tobias Schuerholz⁸, Christian Stoppe¹, Gernot Marx¹, Christoph Thiernemann^{2#} and Lukas Martin^{1#}

¹Department of Intensive Care and Intermediate Care, University Hospital RWTH Aachen, Aachen, Germany

²William Harvey Research Institute, Queen Mary University London, London, United Kingdom

³Department of Drug Science and Technology, University of Turin, Turin, Italy

⁴Interdisciplinary Centre for Clinical Research Aachen, RWTH Aachen University, Aachen, Germany

⁵Institute of Biochemistry and Molecular Biology, RWTH Aachen University, Aachen, Germany

⁶Department of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Jena, Germany

⁷Septomics Research Center, Jena University Hospital, Jena, Germany

⁸Department of Anesthesia and Intensive Care, University Hospital Rostock, Rostock, Germany

#Shared senior authorship and corresponding authors:

Dr Lukas Martin, MD PhD MHBA

Uniklinik RWTH Aachen

Operative Intensivmedizin and

Intermediate Care

Pauwelsstraße 30

52074 Aachen, Germany

lmartin@ukaachen.de

Phone: +49 (0)241 8037606

Prof Chris Thiernemann, MD PhD FMedSci

Queen Mary University London

The William Harvey Research Institute

Centre for Translational Medicine & Therapeutics

Charterhouse Square

London EC1M 6BQ, UK

c.thiernemann@qmul.ac.uk

Phone: +44 (0) 20 78822107

Conflict of interest statement: The authors have declared that no conflict of interest exists.

Supplemental Methods

Renal dysfunction and hepatocellular injury analysis

Renal dysfunction and hepatocellular injury were analyzed in the serum of all mice. At 24 h after surgery mice were fully sedated via 3 l/min isoflurane and 1 l/min oxygen before being sacrificed. Closed cardiac puncture was performed with a G-25 needle where the blood was immediately decanted into 1.3 ml serum gel tubes (Sarstedt, Nümbrecht, Germany). The blood samples were centrifuged for 3 min at 9000 RPM, where the serum was collected and snap frozen in liquid nitrogen and stored at -80°C. The serum was then sent to an independent veterinary testing laboratory (MRC Harwell Institute, Oxford, UK) to blindly quantify serum urea and creatinine known markers of renal dysfunction and alanine transaminase (ALT) and aspartate transaminase (AST) known biomarkers of hepatocellular injury.

Supplemental Tables

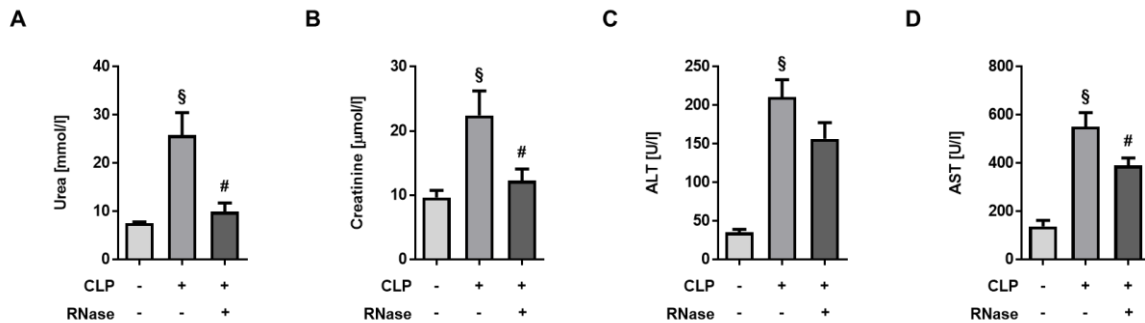
Table 1: Patients' characteristics

	Healthy (n = 10)	Sepsis DD (n = 21)	Sepsis D3 (n = 21)
Age (year) (IQR)	55 (49-74)		63.0 (44-84)
Male sex (number) (%)	7 (70.0)		18 (85.7)
BMI (kg/m²) (IQR)	-		28.1 (20.1-38.4)
Diabetes mellitus (%)	-		6 (28.5)
Creatinine (mg/dl) (IQR)	-	1.2 (0.4-2.9)	1.0 (0.4-3.0)
Hemoglobin (g/dl) (IQR)	-	9.2 (0.0-13.5)	8.8 (7.1-10.8)
Platelets (10⁹ cells/nl) (IQR)	-	237.9 (83.0-553.0)	215.2 (80.0-449.0)
White cells (10⁹ cells/nl) (IQR)	-	15.3 (6.7-27.4)	12.4 (5.9-24.2)
Neutrophil (10⁹ cells/nl) (IQR)	-	81.0 (56.0-92.0)	74.4 (48.0-91.1)
Eosinophil (10⁹ cells/nl) (IQR)	-	1.0 (0.0-9.7)	2.8 (0.0-13.0)
Monocyte (10⁹ cells/nl) (IQR)	-	5.9 (3.0-8.3)	7.8 (2.0-12.6)
Lymphocyte (10⁹ cells/nl) (IQR)	-	7.0 (2.0-15.0)	12.2 (4.1-32.0)
Albumin (g/l) (IQR)	-	12.1 (1.4-28.8)	11.3 (1.5-30.6)
PCT (ng/ml) (IQR)	-	7.2 (0.1-100.0)	5.5 (0.1-76.5)
CRP (mg/dl) (IQR)	-	172.9 (3.9-274.8)	160.6 (36.7-346.1)
Lactate (mmol/l) (IQR)	-	2.2 (0.8-9.4)	1.5 (0.7-2.5)
LDH (U/l) (IQR)	-	313.2 (163.0-548.0)	301.2 (154.0-515.0)
SOFA (points) (IQR)	-	6.7 (2.0-13.0)	4.9 (0.0-11.0)
APACHE II (points) (IQR)	-	15.0 (0.0-29.0)	
LOS ICU (days) (IQR)	-		11.0 (1.0-26.0)
28-day mortality (%)	-		1 (4.8)

Patients' characteristics according to the groups Healthy, Sepsis on the Day of diagnosis (Sepsis DD) and Sepsis three days after diagnosis (Sepsis D3). Categorical and continuous variables are presented as n (%) and median (interquartile ranges, IQR), respectively. BMI = body-mass-index; PCT = Procalcitonin; CRP = C-reactive protein, SOFA = Sequential Organ Failure Assessment score; APACHE II = Acute Physiology and Chronic Health Evaluation II score; MV = mechanical ventilation; LOS = length of stay.

Supplemental Figures

Supplemental Figure 1



RNase 1 treatment of mice with polymicrobial sepsis resulted in an improved renal dysfunction and hepatocellular injury

Serum levels of (A) urea, (B) creatinine, (C) alanine transaminase (ALT) and (D) aspartate transaminase (AST) of sham operated mice and mice with polymicrobial sepsis induced by CLP treated with vehicle or RNase 1 were analyzed (all n = 12). Mean \pm SEM; one-way ANOVA followed by Bonferroni test for multiple comparisons § p < 0.05 vs Sham, # p < 0.05 vs CLP + Vehicle; CLP = cecal ligation and puncture; RNase = ribonuclease 1